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MULTIPLE MOTOR INNERVATION OF THE FROG'S SARTORIUS MUSCLE

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(Received for publication November 25, 1940)

THE FROG'S sartorius muscle owes its place as a classical preparation for the study of muscular activity and myoneural transmission to its well-suited structure, being a thin muscle of long parallel fibres and containing at its pelvic end a region of several millimetres which is free from motor nerve endings. In this paper another peculiar property of the frog's sartorius is described, *viz.* that practically all its fibres are provided with at least two motor nerve endings situated in discrete regions, and often concentrated within definite narrow zones, of the muscle.

METHOD

The experiments were made, during January–May 1940, at 20–30°C., on isolated sciatic-sartorius preparations of Australian frogs (*Hyla aurea*). The animals were kept in the laboratory in a cold store, at about 14°C. Their sartorius muscles are about 35 mm. long, delicate and thin, and resembling in size the sartorius of European *Rana temporaria;* they remained in good condition for many hours during the experiment, even at high temperature. As a rule, the preparation was soaked for an hour in oxygenated Ringer's solution before use.

The preparation was mounted in a moist paraffin wax chamber on platinum electrodes the position of which could be adjusted from the outside. The Pt-wires were attached to ebonite rods sliding, in parallel with the longitudinal axis of the muscle, in ebonite tunnels which were fixed in the paraffin block at either side of the chamber.

The muscle was stimulated, directly or through its nerve, by break induction shocks delivered from a Lucas pendulum. The action potential of the muscle was recorded with a 3-stage condenser coupled amplifier and cathode ray oscillograph. With a rectangular voltage input, the preparation being replaced by moist filter paper, the oscillograph deflection reached 90 per cent of its maximum in 0.1 msec., and declined to $\frac{1}{2}$ in 0.2 sec.

When recording from the sartorius muscle, "lead artefacts" such as described by Bishop, Erlanger and Gasser (1926) and by Bishop and Gilson (1929) are sometimes encountered. (i) When the leads are applied at either side of the nerve entry, fluid and tissue rests attached to this region act as a "false lead" which complicates the record by introducing a small diphasic wave. (ii) A positive initial wave preceding the period of negativity was sometimes recorded when leading from the extreme end of the muscle, or when using submaximal stimulation (cf. Bishop and Gilson, 1929).

In some cases the twitch tension was recorded with an optical isometric myograph, the deflection being read on a scale.

RESULTS

1. SHAPE OF THE ACTION POTENTIAL OF THE FROG'S SARTORIUS

The distribution of motor nerves in the frog's sartorius has frequently been described (*e.g.* Pézard and May, 1937) and is shown diagramatically in Fig. 1. If recording leads are placed on the pelvic part of the muscle (Fig. 1) and the nerve is stimulated by a maximum induction shock, one would expect to observe a prolonged and complicated action potential made up by

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individual waves which originate in the various junctional regions and arrive in succession at the pelvic ends of the muscle fibres. Considering (i) that the sartorius consists largely of long fibres running from end to end (cf. section 5), (ii) that the motor endings are scattered over a distance of about 2 cm. and (iii) that the muscle impulses travel at a speed of about 2 m/sec., at 25°C, one might expect to obtain a very complex potential wave with individual spikes separated by as much as 10 msec. It was rather surprising, therefore, to find in a normal muscle, as the result of a maximal nerve stimu-

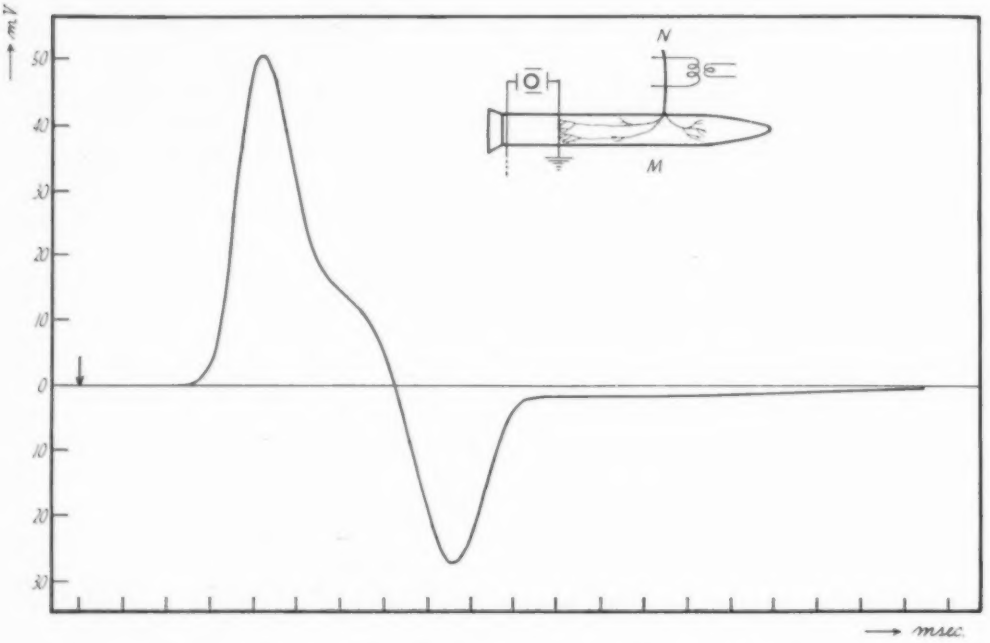

FIG. 1. Diphasic action potential of frog's sartorius at 22°C. Leads at 8 and 0 mm from pelvic end. Nerve stimulus marked by arrow. Inset: Diagram of sartorius (M) with nerve (N) and stimulating and recording leads. Approximate position of main innervation zones is indicated.

lus, a single spike (Fig. 1, 2) as large as, and not longer than, that obtained by maximum direct stimulation (*i.e.* about 50–60 mV. and 4–5 msec. respectively). Obviously the expected late waves starting on the other side of the nerve entry had failed to arrive at the recording electrode, while apparently, in a majority of the muscle fibres, impulses had been initiated nearby. A similar result was obtained when recording from the tibial part of the muscle.

The action potential wave is not always smooth and synchronous as in the cases of Fig. 1 and 2a. Often it has two or three distinct humps (Fig. 2b) indicating that the points of origin of the impulses in different fibres are scattered over a few mm. While the shape of the action potential thus varied from muscle to muscle, and from point to point in each preparation (see

section 3, p. 217 below), the absence of delayed spikes which might have come from the distant parts of the muscle was consistent.

A possible explanation of this phenomenon might be the presence in

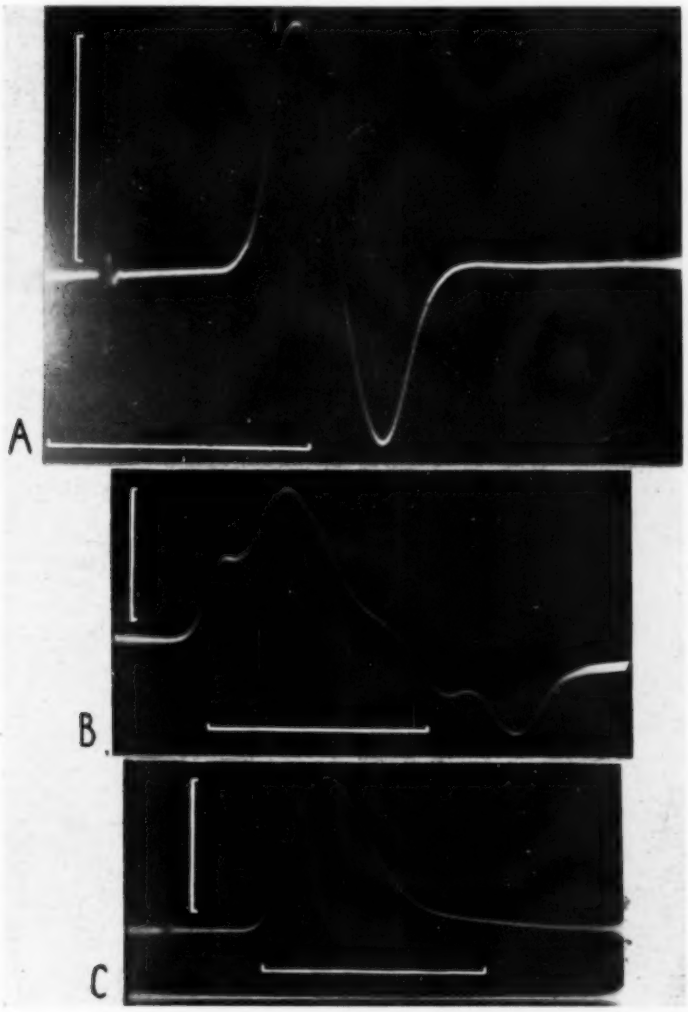

FIG. 2. Action potentials of sartorius muscles. Indirect stimulation. *a*. Diphasic. 20°C. Leads at 7.5 and 4 mm (from pelvic end). *b*. Diphasic. 18°C. Leads at 10 and 0 mm. *c*. Monophasic (by application of isotonic KCl to pelvic end). 18°C. Active lead at 12 mm. Time scale: 5 msec. Volt scale: 50 mV.

most fibres of multiple motor nerve endings, concentrated in discrete "neural" zones. In this way impulses originating at nerve endings far away from the amplifier leads would collide during conduction with those coming from the more proximal junctions, and so could not be recorded. This suggestion seemed to contrast with the current view (Kulchitsky 1924, Fulton

1926) *viz.* that multiple innervation of individual muscle fibres does rarely, if ever, occur. In several ways, however, clear-cut evidence for the correctness of this explanation was obtained.

2. EVIDENCE FOR MULTIPLE INNERVATION

a. Removal of one nerve branch

The nerve supplying the sartorius divides into two main branches, a pelvic and a tibial branch, immediately before entering the muscle. If, for example, the pelvic branch is cut, the spike previously recorded from the

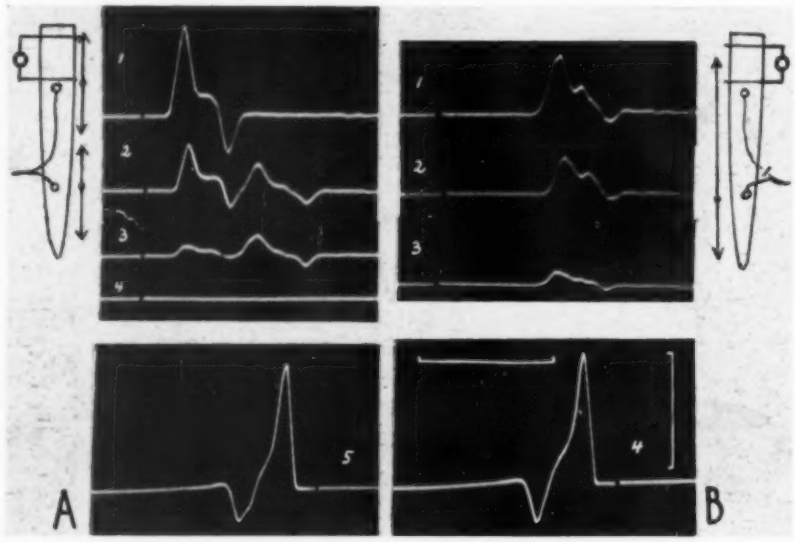

FIG. 3. Effects of partial denervation and of submaximal stimulation. Sartorius, 38 mm long, at 27.5°C. *A*. Intact nerve supply. A, 1–4: Leads at pelvic region (12 and 2 mm). Strength of nerve stimulus, successively from above, in relative units: 123 (maximal), 103, 100, 99. A, 5: Leads at tibial region (28 and 35 mm; record runs from right to left). Maximal nerve stimulus. *B*. After pelvic branch has been cut (cf. diagram). B, 1–3: Leads at pelvic region (10 and 1 mm). Strength of stimulus: 117 (maximal), 103, 100. B, 4: Leads at tibial region. Maximal stimulus. Time scale: 10 msec. Volt scale: 30 mV.

pelvic end (Fig. 3 and 4) disappears and, in its stead, a delayed spike is observed reaching the pelvic electrodes after a latency of 7 msec., instead of 2 msec. (Fig. 3, B). Obviously, those impulses which start in the tibial part of the muscle, supplied by the intact nerve branch, and which previously had been stopped on their way by collision with the pelvic impulses are now able to travel unhindered to the pelvic end of the muscle. Action potentials recorded from the tibial "intact" portion remain unaltered.

The delayed spikes reached only 20–35 mV in amplitude, *i.e.* about 50–70 per cent of the early spike, an effect attributable to the increase in temporal dispersion. The difference between the spike latencies before and after nerve section varied between 5 and 7 msec. With a conduction velocity of

2–2.5 m/sec., therefore, it appears that many fibres are innervated in 2 discrete "zones" of the muscle, about 12–15 mm. apart.

It is clear that delayed spikes must appear whenever the collision of muscle impulses is abolished or rendered incomplete. This can be achieved in various ways, *e.g.* by cutting one nerve branch as above, by *submaximal*

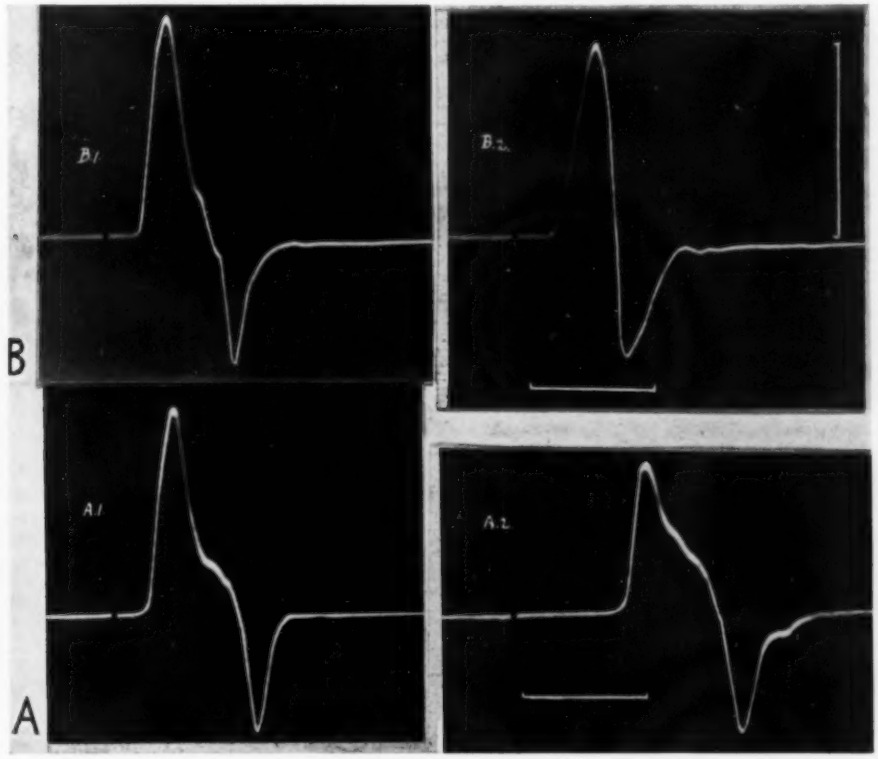

FIG. 4. Effect of partial denervation. 16.5°C. *A*. Half-denervated sartorius muscle. Pelvic branch had been cut 16 days before the experiment. Maximal nerve stimulus: A, 1: recording from tibial; A, 2: from pelvic region. *B*. Other sartorius of same frog. Intact nerve supply. B, 1: tibial; B, 2: pelvic recording. Time scale: 10 msec. Volt scale: 50 mV.

nerve stimulation, by partial curarization, and finally by interposing a direct stimulus at a suitable time interval.

b. Submaximal stimulation of the motor nerve

Figure 3 shows action potentials of the sartorius muscle (i) to maximal, (ii) to submaximal stimulation of the nerve. It so happens that in the latter case, the impulses set up at the pelvic and tibial junctions respectively are travelling largely in separate fibres and, therefore, a discrete late spike is recorded, which is not present when all nerve fibres are excited by stronger stimuli. The phenomenon is not always marked; it depends largely upon a chance arrangement, *viz.* upon the presence and frequency of individual

muscle fibres which happen to be supplied, at their respective pelvic and tibial junctions, by *separate* axons of *different* excitability.

If one branch of the nerve is cut, as above, then with all strengths of stimuli only the early spike is recorded on the intact side, and only the late spike on the denervated side.

c. Partial curarization

A condition similar to submaximal nerve stimulation is obtained by weak curarization of the muscle. For this, the preparation was soaked in Ringer's

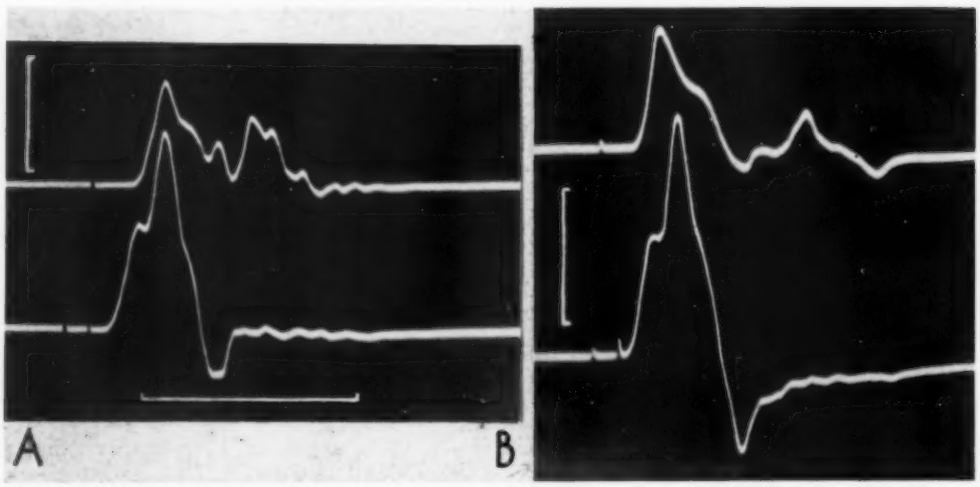

FIG. 5. Partial curarization. Upper records: Single, lower records: double maximal nerve volleys. A. Surface application of curarine (3μ mol.l.) to pelvic part. 27.5°C. B. Muscle soaked in 1μ mol./l. curarine. 26°C. Time scale: 10 msec. Volt scale: 20 mV.

solution containing 1 μmol. curarine per 1 litre. In this way a stage of incomplete neuro-muscular block is attained in which, in many muscles fibres, one junction happens to be blocked, while the other is still functioning, so that many impulses can run along to the other side of the muscle without colliding. Figure 5 shows an example of this kind. The normal action potential is reduced in size and split into 2 discrete spikes, about 7 msec. apart, by weak curarization. If the "distal" nerve branch is cut, the late spike disappears.

There is another way of abolishing the late spike in a partially curarized preparation, namely by sending a second nerve volley down to the muscle at a brief interval after the first. Figure 5 shows that a second stimulus to the nerve applied 1.3 msec. after the first, produces a considerable addition to the early spike, and practically abolishes the delayed spike. On account of neuro-muscular facilitation (cf. Bremer, 1927) the second volley excites at nearly all those junctions which had failed to transmit the first impulse and so the late spike is eliminated by collision as in normal muscle.

Incidentally the second volley has been discharged so early in the refractory period of the muscle that no second impulse could be set up in those fibres which were activated by the preceding volley.

d. "Antidromic" stimulation

The presence of multiple innervation can be demonstrated in another way. The muscle is excited directly by a maximum shock to its pelvic end (*M* stimulus), and a little later, when the muscle volley has travelled beyond the pelvic junctions, a nerve volley (*N*) is sent down. The following situa-

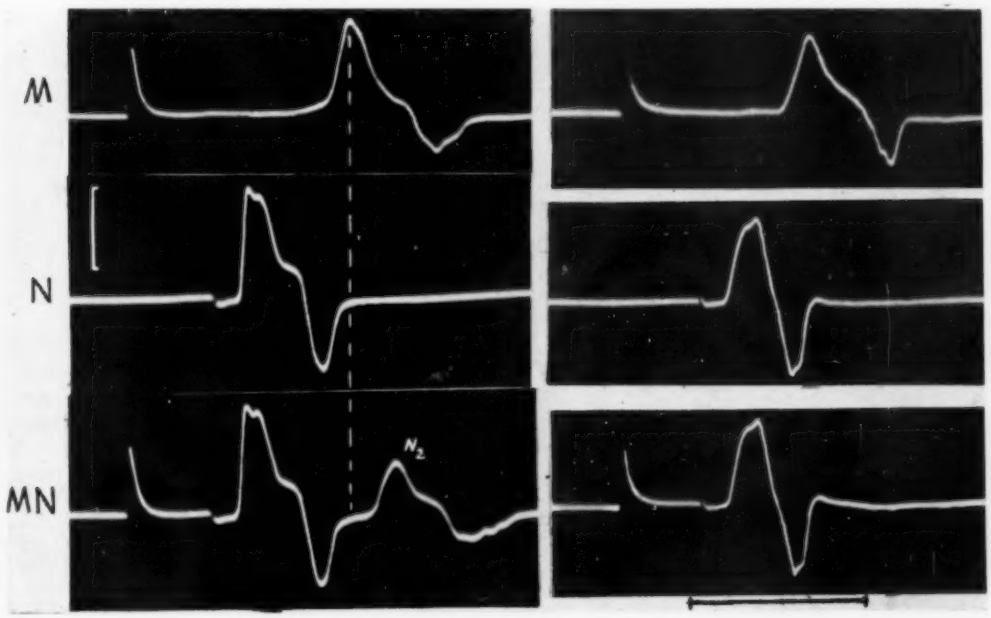

FIG. 6. "Antidromic" stimulation. Muscle 34 mm long. 26.5°C. Leading at tibial region (24 and 32 mm). Direct stimulation at pelvic end (cathode at 3, anode at 1 mm). M, direct stimulus. N, nerve stimulus. MN, combined stimuli, N 4.9 msec. after M. Left: intact nerve supply. Right: pelvic branch cut. The M spike collides with, and is blocked by, the N spike (Right, MN), but makes way for a late spike (N_2), coming from the pelvic region (Left, MN). Note the different timing of M and N_2, and the disappearance of N_2 after the pelvic nerve branch has been cut. Time scale: 10 msec. Volt scale: 20 mV.

tion occurs: the muscle volley set up by direct stimulation (referred to below as the "antidromic" volley since it travels from the nerve-free end towards the junctional region) collides, somewhere near the nerve entry, with the impulses coming from the tibial junctions. In the wake of the antidromic volley, the muscle is still refractory, but farther back at the pelvic junctions it has recovered and so another muscle volley, initiated by the nerve, follows behind the antidromic volley and travels unopposed down to the tibial end, where it is recorded (Fig. 6). The antidromic volley has cleared the way for the pelvic impulses.

The size and, within certain limits, also the timing of the late "pelvic"

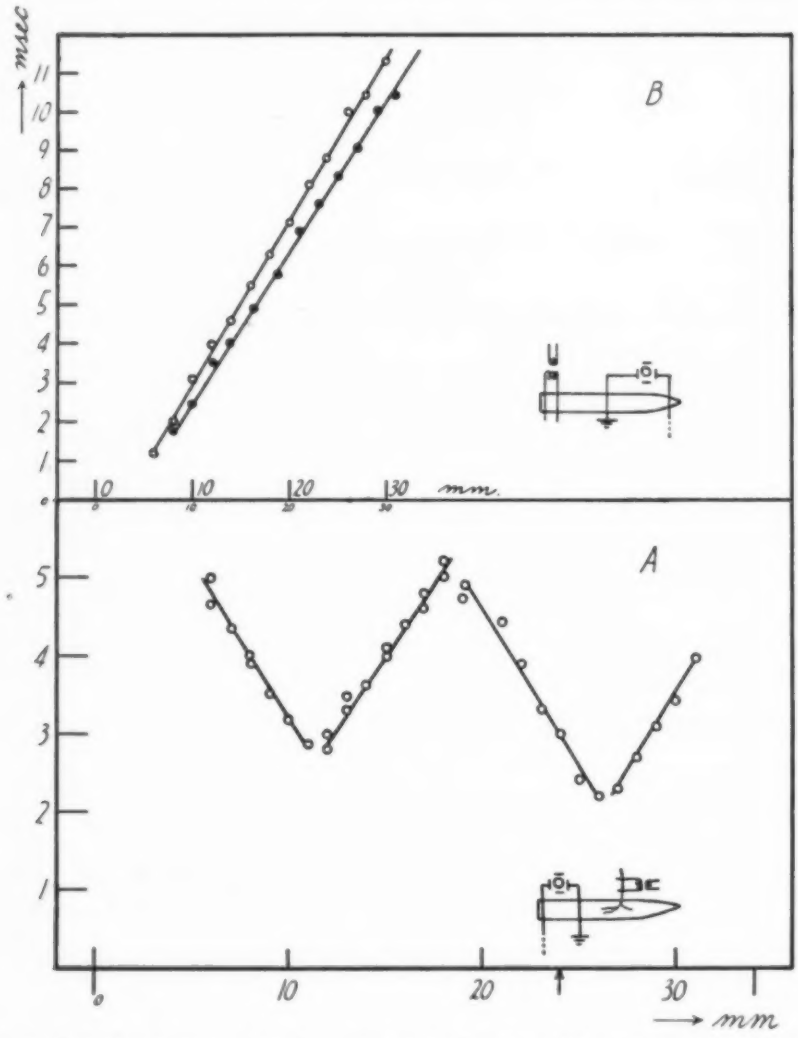

FIG. 7. Relation between shock-peak interval and position of recording lead. 27.5°C. *A*. Nerve stimulation. Position of nerve entry marked by arrow. Length of muscle 34 mm. *B*. Direct stimulation. B, 1. Normal. B, 2. After 10μ mol. curarine. With B, 2, the muscle was stretched to a 6 per cent greater length which probably accounts for the apparent slight increase (10 per cent) in conduction velocity. Abscissae: position of earth lead on muscle, in mm from pelvic end. Ordinates: shock-peak time in msec.

spike depends upon the interval between the *M* and *N* stimuli. If the nerve volley is fired too soon, or too late, *i.e.* before the pelvic, or after the tibial, junctions have recovered from the antidromic volley, the late spike fails to appear. With intermediate intervals, the size of the late spike becomes larger and its latency smaller, as the antidromic volley and the recovery from it, proceeds towards the tibial junctions.

If the pelvic nerve branch is cut, the late spike is abolished (Fig. 6, Right, MN).

3. LOCALIZATION OF MULTIPLE MOTOR ENDINGS

The preceding experiments show the presence of an extensive multiple innervation in the sartorius muscle. Further information, however, is required regarding (i) the number (two or several) of motor nerve endings in individual fibres and their exact position, (ii) the fraction of the muscle fibres concerned.

If the position of the earthed electrode (Fig. 7) is varied along the muscle, systematic changes in shape, size and timing of the spike are observed (Fig. 7–10).

In the following, the position of the earth lead on the muscle is given in millimetres distance from the pelvic end. The grid lead is applied to "0 mm," unless stated otherwise.

From about 8 mm to the pelvic end, the impulses are conducted at a uniform rate of about 2 m/sec. This applies to both indirect and direct stimulation, and to normal as well as curarized muscle (cf. Göpfert and Schaefer, 1938). It is merely a confirmation of the well known absence of motor nerves at the pelvic end.

Beyond that region, one must distinguish between the *beginning* and *summit* of the action potential wave. The start of the action potential ("shock-start" delay) varies only slightly with the position of the recording electrode. Since some motor endings are scattered usually over the whole of the muscle except the pelvic and extreme tibial end (Pézard and May, 1937), the shock-start delay, beyond 6 or 7 mm., depends only upon conduction in the intramuscular nerves which is about 10 times faster than conduction in the muscles fibres (cf. Göpfert and Schaefer, 1938). Thus the shock-start delay is a minimum at the point of nerve entry, increases slightly, at a rate of about 0.05 msec./mm towards the end

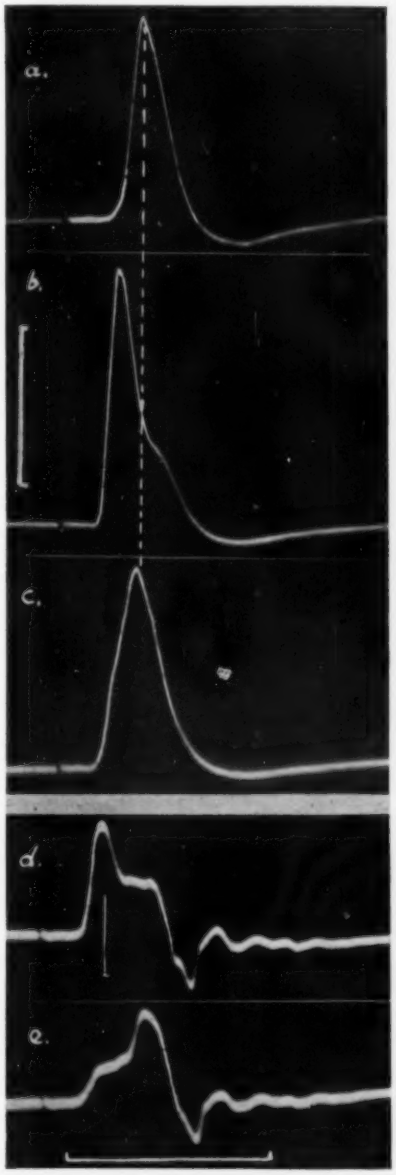

FIG. 8. Changes in form and timing of spike recorded at different positions. *a-c*: 22°C. "Nearly monophasic" (KCl at pelvic end). Positions of active lead, successively from above: 7, 10, 14 mm from pelvic end. Minimum latency at 10 mm. *d-e*: 27.5°C. Diphasic. Position of earth lead at 12 and 19 mm respectively. Time scale: 10 msec. Volt scale: 30mV.

of the muscle until the nerve-free part is reached; from there onwards, it increases at a 10-fold rate.

However, the delay of the summit of the action potential ("shock-peak" delay) varies in a very different fashion (Fig. 7, 8, 10). Figure 7, for example, shows two sharp minima at 11–12 and 26–27 mm, and a maximum shock-peak delay at 18–19 mm, not far from the nerve entry. Obviously, in this

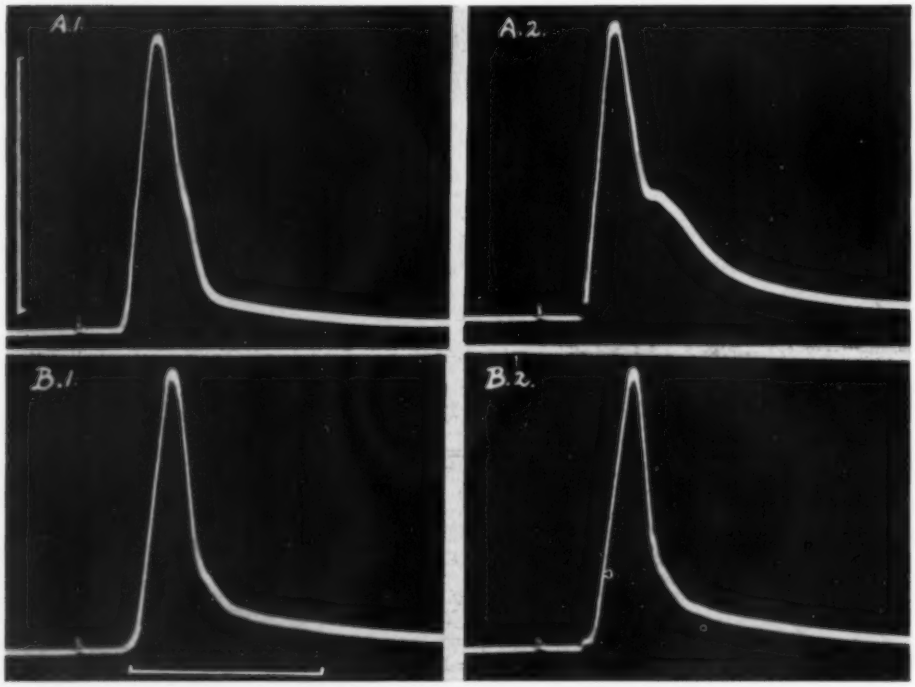

FIG. 9. Effect of early second nerve volley. Monophasic spikes. 18°C. *A*. Position of earth lead at 10 mm. A, 2: Second nerve volley, after 2.5 msec., adds non-conducted "end-plate potential." *B*. Position at 12.5 mm, *i.e.* 2.5 mm nearer the nerve entry. No noticeable addition by second nerve volley (measurement shows an addition which is about 1/30 of that at 10 mm). The spike peak at 12.5 mm is 1.6 msec. later than at 10 mm, corresponding with a conduction velocity of 1.6 m/sec. Time scale: 10 msec. Volt scale: 50 mV.

particular muscle there were two main "innervation zones", at 11 and 26 mm, where volleys of impulses were set up in many muscle fibres proceeding to both sides at a speed of 2.5 m/sec. It is possible to follow them on their way because the position of the spike summit depends, not so much upon the presence of scattered junctions, but upon the arrival of a synchronous volley. It is clear, therfore, that while some motor endings are distributed over the whole length between 11 mm and 27 mm, large fractions are concentrated at definite "neural" zones. The picture is not always as simple as in Fig. 7, usually there are several centres of innervation (see *e.g.* Fig. 10).

These observations have been confirmed by recording the non-propagated potential changes set up in the junctional region of the muscle fibres by a blocked motor nerve

volley (see Eccles, Katz and Kuffler, 1940). These "end-plate potentials" are found only in restricted "neural" zones of muscle, and their positions agree with those of the minimum shock-peak intervals (cf. Fig. 9).

Taken together with the previous evidence (Section 2) it can be concluded that the two "neural" zones shown in Fig. 7, A separated by a distance of

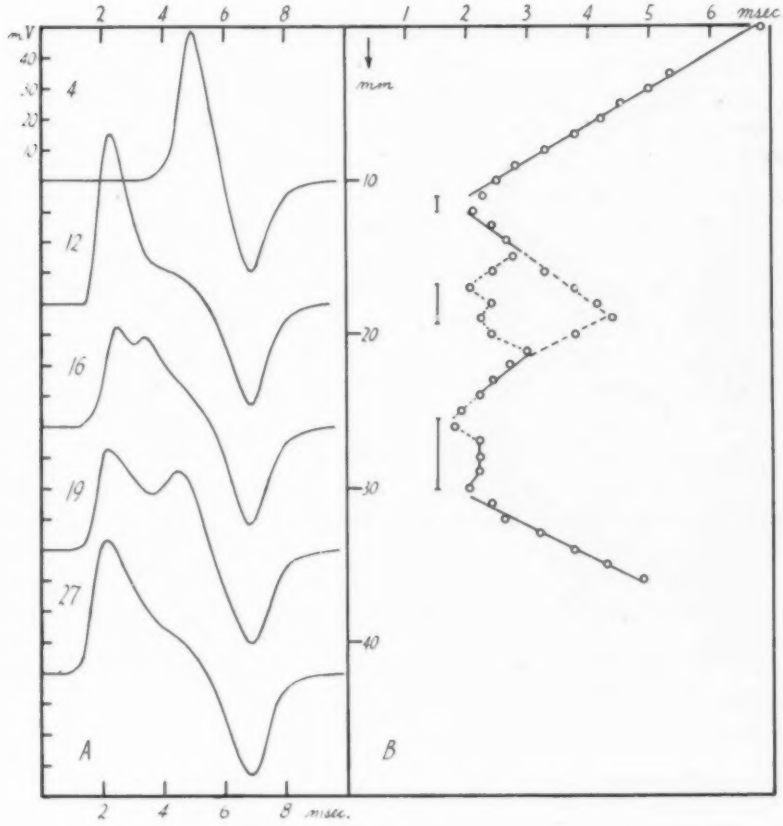

FIG. 10. Changes in timing of spike at different positions. 26°C. A. Diphasic spikes. Earth lead (successively from above) at 4, 12, 16, 19, 27 mm from pelvic end. Grid lead at pelvic end. Volt scale: 10 mV marks. Abscissae: interval after nerve stimulus in msec. B. Ordinates: Position of earth lead on muscle, in mm from pelvic end. Abscissae: Shock-peak interval in msec.

15 mm, belong to the same muscle fibres. Figure 7 shows also that many fibres are innervated at two regions *only*. Otherwise it would not have been possible to follow the conduction of the spike summit from the 2 "neural" zones to the point of collision at 18 mm. On the other hand, there is no doubt that a good many motor endings are distributed over the intermediate distance between 11 and 27 mm. They might belong to separate muscle fibres, not innervated at the 2 main zones, or they might be additional motor endings on the same fibres.

4. NUMBER OF MULTIPLE MOTOR ENDINGS

There is good evidence that these intermediate motor endings constitute an additional (third, or even fourth) nerve supply for a considerable part of the muscle fibres. This is made probable by the high degree of synchronization and the great amplitude of the spikes near the pelvic and tibial ends (Fig. 1, 2). This, together with the fact that most fibres run through the whole length of the muscle (cf. section 5 below) suggests that nearly all fibres are innervated near by, and that the scattered endings are probably additional junctions in the same fibres.

More definite evidence is obtained by comparing the concomitant changes of timing, form, and amplitude of the action potential at different points. An example is shown in Fig. 10. There are 2 main innervation centres, at 11–12 and 26–30 mm, the former being more concentrated, and, therefore, giving rise to a larger and more synchronous spike than the latter. In the intermediate region, the spike is reduced in amplitude and split up into 2 components, (i) an early wave, arising from new myoneural junctions near the recording lead (at 17–19 mm), and (ii) a delayed "hump" due to conducted muscle impulses coming from the main innervation centre. The first group (i) of impulses apparently collides with part of the synchronous volley coming from the main centre, so only a reduced remainder (the delayed hump, ii) is conducted through. Group (i), therefore, belongs to fibres which have 3 (possibly even more) discrete motor nerve endings, while group (ii) has only 2 separate innervation zones.

5. FRACTION OF THE MUSCLE SUPPLIED BY MULTIPLE MOTOR ENDINGS

While the phenomenon of multiple innervation as such was easy to demonstrate, a quantitative estimate of the fraction of the muscle fibres concerned is more difficult to obtain. The very small size, or complete absence, of any "late spike" in the normal action potential (Fig. 1, 2) shows that at least 95 per cent of the fibres which reach through the distance between the 2 main neural zones, *i.e.* through about 60 per cent of the muscle length, *have* multiple innervation. The problem is, therefore, reduced to the question what fraction of the muscle fibres runs through this intermediate length.

There is evidence that a great majority of the muscle fibres runs through nearly the entire length of the sartorius.

If either end of the completely curarized muscle is stimulated by maximal induction shocks, and the action potential recorded at various distances away from the stimulated region, there is a marked progressive decline in the amplitude of the spike, but little change in its potential-time area (Table 1). This indicates that most fibres run from end to end, and that the progressive decline of spike height during conduction is merely due to different velocities of the impulses in different fibres.

Table 1

Curarized muscle stimulated directly at one end. Position of earth lead at 1/4, 2/4 and 3/4 of muscle length; grid lead at the other end. The potential-time area, in each record, was integrated up to an arbitrary constant interval reckoned from the peak of the spike and chosen so that the diphasic potential change (at the grid lead) did not interfere. Mean values and extreme variations of 5 experiments.

	Recorded at			
	1/4	2/4	3/4	(4/4) of muscle length.
Amplitude	100	75(67–82)	62(48–70)	(61(42–68)[x])
Area	100	90(84–94)	92(80–109[xx])	

[x] Approximate size of diphasic change at the grid lead.

[xx] This exceptionally large value involves probably a large negative after-potential at this region.

Furthermore, the mechanical twitch tension to a maximum nerve stimulus is only slightly reduced (by 6–12 per cent, mean 10 per cent), if one of the 2 nerve branches is cut. This result may not be very significant, since for various reasons, the mechanical twitch tension cannot safely be considered as a proportional index of the number of active fibres, but it does not disagree with the evidence obtained by electric recording.

In a few cases the fibres of the sartorius muscle were isolated under a low-power binocular microscope, after the frog's leg had been boiled, as described by Buchthal and Lindhard (1939). The great majority of the fibres were found to run from end to end. In one case, out of 100 fibres, only 5 did not reach through the entire length. Short fibres, of only a few mm. length, as described by Mayeda (1890) were not observed. It may be that this discrepancy is due to the different kind of animals used (Buchthal and Lindhard *e.g.* mention that fibre lengths differ in the sartorius muscles of *Rana esculenta* and *temporaria*), or that the reported short fibres were fragments produced by the dissection.

As a whole, it seems safe to conclude that the great majority of the fibres runs through practically the entire length of the muscle, and that, therefore, nearly the whole muscle is provided with multiple motor nerve endings.

Discussion

The question of a multiple motor nerve supply in skeletal muscle has previously been discussed, but its existence, to any great extent, has not been accepted (see, however, Cattell 1928). According to Fulton (1926) and Wilkinson (1929) multiple motor endings are a rare phenomenon, which indicates that the frog's sartorius muscle must be an exceptional case. Kulchitsky (1924), however, stated that no double motor supply could be found, with methylene-blue staining, in the frog's sartorius muscle, which is in obvious disagreement with the present experiments. It is difficult to see how Kulchitsky arrived at this conclusion since in none of his reported observations did he follow an individual muscle fibre for more than a few millimetres.

In view of these statements, we were rather astonished to obtain evidence for a very extensive multiple motor supply in the frog's sartorius, but were no less surprised by a subsequent discovery, *viz.* that corresponding histological evidence had been presented already by Sandmann (1885) in a paper which apparently escaped notice in the more recent literature. Sandmann found, with gold-staining of isolated muscle fibres, that most fibres of the frog's sartorius have 2 or 3 discrete motor nerve endings, some have even more (up to 6), while single endings are only exceptionally observed. He refers also to the presence of distinct "innervation zones."

Sandmann also dealt with the question of "pluri-segmental" innervation of the sartorius muscle. He found that, after severing one of the supplying motor roots, only a few fibres contained a degenerated *and* an intact nerve ending, while most fibres had either completely retained, or lost, their motor supply. More recently, however, Cattell (1928) concluded from a comparison of the mechanical responses with separate and combined stimulation of the motor roots that about $\frac{1}{3}$ of the sartorius (varying between nil and 73 per cent receives "bi-segmental" innervation.

The multiple motor supply may have some functional significance: for as a consequence the impulses need to travel only about $\frac{1}{4}$ of the fibre length to throw the whole muscle fibre into activity, and so external force would be exerted at a somewhat higher rate (see Gasser and Hill, 1924; Hill, 1938). However, the time saved in the spread of the excitation wave is small (about 5 msec. at 25°C), hence it remains doubtful whether much is gained by it.

The peculiar arrangement of the motor endings has, however, a definite experimental interest; it is possible, for instance, to obtain preparation where despite the wide distribution of myoneural junctions, practically the whole of the muscle fibres is innervated in a narrow zone, of a width of one mm, adjoining the nerve-free pelvic end. Such a preparation offers a number of important advantages for the study of neuro-muscular transmission, in particular of local electric potential changes at the myoneural junction. It is intended to report, in a later paper, a series of observations obtained in this way.

Summary

Experimental evidence is described for the existence of multiple motor nerve endings in most fibres of the frog's sartorius muscle.

The electric response of the muscle, to a maximum nerve stimulus, is recorded. In both, pelvic and tibial, regions a large, synchronous spike, of about 50 mV and short latency (1–2 msec.) is usually obtained. This seems incompatible with the facts that the motor nerve endings are distributed over a length of about 2.5 cm. and that most of the sartorius fibres run through the whole length of the muscle (cf. section 5).

The apparent discrepancy is due to the presence of multiple (2 or 3) motor nerve endings on most fibres. Muscle impulses are set up simultaneously at these junctional regions of each fibre; only the impulses which arise nearest to the amplifier leads are recorded, since those more distal are blocked by collision.

This can be demonstrated by various means, *e.g.* by cutting one of the two main nerve branches, by partial curarization, by submaximal nerve stimulation, and by combined application, at suitable time intervals, of 2 stimuli (i) to the motor nerve and (ii) to the nerve-free pelvic end of the muscle. By all these procedures, some myoneural junctions are put out of action and, therefore, some of the "distal" impulses (arising far away) can travel unhindered to the recording leads. In this way, a part or the whole of of the normal, synchronous and early, spike potential is abolished and replaced by a discrete potential wave of long latency.

From the variations in size, shape and latency of the spike along the muscle it is often possible to locate 2 or 3 discrete, narrow, "neural " zones, which contain the majority of the myoneural junctions.

We are indebted to Dr. J. C. Eccles for helpful discussion and criticism.

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FAST CENTRAL FIBERS IN FISH

PROPERTIES OF MAUTHNER AND MÜLLER FIBERS OF MEDULLOSPINAL SYSTEM[1]

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IN TELEOSTS and urodeles two giant nerve cells (cells of Mauthner) are to be found, one on either side of the medulla oblongata at the level of the acoustic decussation (Mayser, 14; Beccari, 3; Bartelmez, 1). In representative teleosts, such as the common catfish of the genus *Ameiurus*, the extraordinarily large sheathed axons of these two cells may be identified through the medulla and most of the spinal cord. They decussate shortly after their origin, and as they pass caudally they give off many short unbranched collaterals which terminate synaptically upon the primary motor neurones of the medulla oblongata and spinal cord (Tagliani, 17; Beccari, 3; Tiegs, 18). The lateral dendrite of each Mauthner cell is exceptionally long and thick and at its terminus is enveloped by a sheaf of synaptic clubs which are the endings of a group of root fibers of the VIIIth cranial nerve (Beccari, 3; Bartelmez, 1; Bartelmez and Hoerr, 2; Bodian, 6). According to Beccari these VIIIth root fibers are of saccular origin.

Bartelmez concluded from his fundamental work on the motor tegmental nucleus of fishes that the Mauthner cells are specialized components of a group of large nerve cells lying in this nucleus and previously homologized by Edinger (8) with the Müller cells of cyclostomes. These cells, of paired paramedian distribution, lie dorsal to the lateral longitudinal fasciculus (*llf*) at the level of the acousticolateral nuclei. They are presumably activatable through intercalary channels originating in the central nuclei of the VIIIth cranial nerve. Their myelinated axons are among the largest in the medulla oblongata, and after decussation pass through the median longitudinal fasciculus (*mlf*) to the ventromedial area of the spinal cord. It is generally believed that the Mauthner cells are differentiated from the rest of this group by short-circuiting of the intercalary channels to them, so that their lateral dendrites receive impulses directly from bipolar acoustic ganglion cells whose peripheral terminals distribute to the saccule of the internal ear.

The present investigation deals with the properties of the fibers passing from the *mlf* of the medulla through the spinal cord. Knowledge of their properties is a necessary prelude to the study of their synaptic activation.

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METHOD

In adult catfish (*Ameiurus melas* and *nebulosa*), the potentials propagated down the spinal cord following stimulation of the *mlf* in the medulla oblongata were recorded by means of a differential amplifier and a cathode ray oscillograph (mean length of cords, 140 mm.; conduction distances used, 50–115 mm.). Each fish was kept in water at 10–15°C. throughout the experiment as well as for 24 hours before, and during the experiment was fixed in such a position that normal respiration could occur but no water could enter the operative opening. The dissection was limited to opening the cranial cavity and exposing the vertebral column. At the temperatures used, minimal amounts of chloretone dissolved in the water provided sufficient anesthesia. Respiration usually continued to the end of the experiment.

Steel needles insulated to their tips (16–100μ in diameter) were used for both recording and stimulating. Paired stimulating electrodes (1.5–2 mm. apart) were inserted vertically into the medulla on either side of the *mlf* to a depth decided upon from previously determined data and measured micrometrically, or a single needle was used as cathode in the *mlf* and a large needle as anode in the adjacent cartilage. One recording electrode was thrust into the ventromedian raphe of the cord (arrows, Fig. 7 to 10), and the other was either in the cord 4–5 mm. caudal to the first, or in the vertebral cartilage 10–20 mm. caudal. The timing of the oscillograph trace sweep with the discharge of the stimulating thyratrons was controlled electrically (Loeffel, 13). As far as possible, stimulation was confined to a range which was subthreshold for muscle potentials; when muscle potentials were present they were recorded separately from the cord fiber potentials by means of electrodes inserted into the muscle near the recording electrodes in the cord. As all muscle potentials occurred after a delay of at least 5 msec. following the stimulus, they were readily distinguished from the prompter cord fiber potentials in the records from the cord.

Several procedures were used in securing histological controls upon the experimental studies. In accordance with the suggestions of Bartelmez (1), Bartelmez and Hoerr (2), and Bodian (6), from several fish perfused with chilled formol chrome sublimate, the medulla and spinal cord were removed rapidly, mordanted in potassium dichromate, and sectioned at 5μ. The sections of this material, stained with Azan Mallory, are designated as "cytologically prepared." Myelin sheaths were stained by the Kultschitzky method applied upon Weigert mordanted material, or by 2 per cent osmic acid. For axon preparations the "short protargol" method of Davenport, McArthur and Bruesch (7) was followed. The traces of recording or stimulating electrodes were examined in serial 20μ sections stained in cresyl violet.

Histological Observations

1. *Anatomical relations of the median longitudinal fasciculus.* Figure 1 illustrates the fiber composition of the medulla oblongata of Ameiurus at the most rostral level of stimulation. This, hereafter designated as the "rostral level," corresponds to line 1 of the inset figure, an outline sketch of a dorsal view of the medulla and cerebellum adapted from Bartelmez (1). Figure 1 is a photomicrograph from a 5μ transverse Weigert series through the brain of an Ameiurus of 66 mm. cord length. The central rectangle is located within the densest aggregation of large myelinated fibers in the medulla. These fibers are coursing longitudinally and the aggregation includes the *mlf* and the *llf*. The rectangle is set asymmetrically so that it divides the *mlf* from the *llf* on the side toward the inset figure and includes the medial part of the *llf* on the side away from it. At the level of line 1 the *mlf* is divided into supra- (*sc*) and infracommissural (*ic*) bundles by the acoustic commissure (*ac*). The arrow under *D* (dorsal) in the figure indicates the position of the two Mauthner fibers in the *sc* bundle. These fibers originated from the Mauthner cells a few sections rostrally and decussated a few sections caudally.

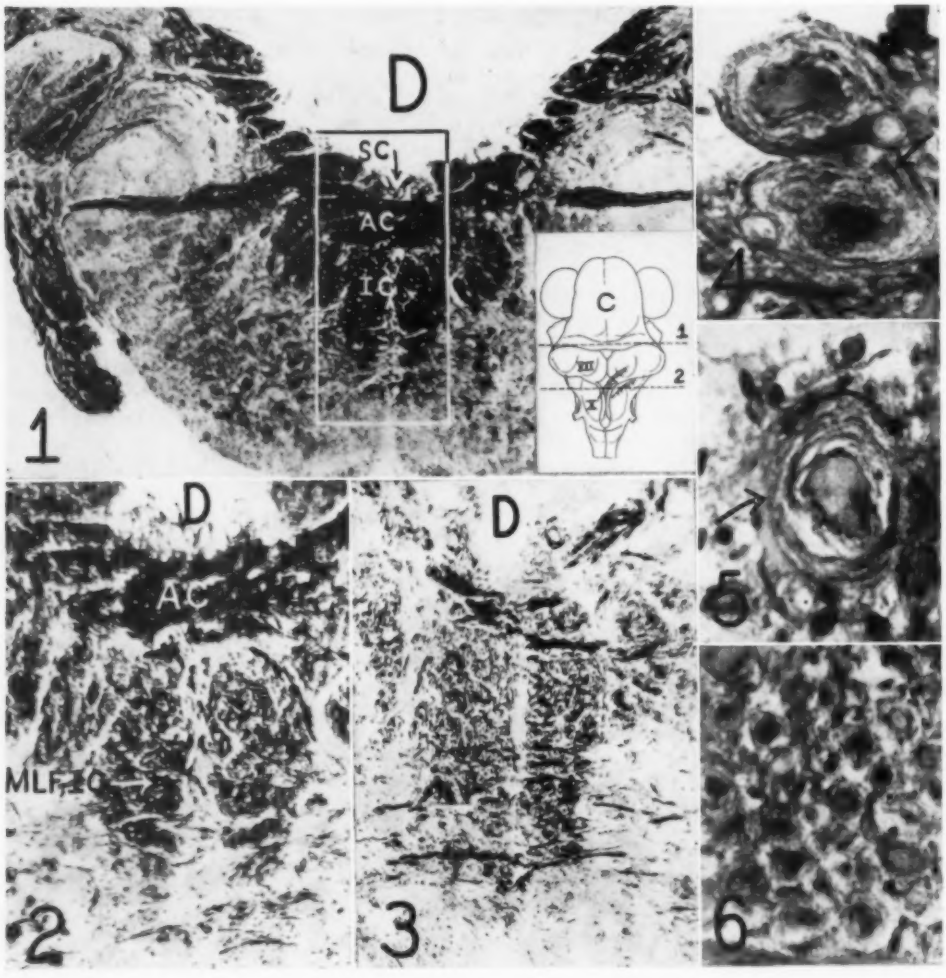

FIG. 1–6. Photomicrographs illustrating the situation and appearance of Mauthner and Müller fibers in the medulla of *Ameiurus*. Figs. 1–3 selected from material fixed by perfusion in formol bichromate, Weigert mordanted, and colored with Grubler's Basiline. Figs. 4, 5 and 6 selected from a medulla fixed by perfusion with formol chrome sublimate; sections colored with Azan Mallory.

FIG. 1. (×29) Cross-section of the medulla at the "rostral level." The median (*mlf*) and a portion of the left lateral longitudinal fasciculus are enclosed within the rectangle. The two Mauthner fibers (arrow) are situated in the supracommissural (*sc*) bundle just dorsal (D) to the acoustic commissure. The inset is an outline sketch of the medulla and cerebellum of *Ameiurus* viewed from its dorsal surface. *C*, cerebellum; *VII*, lob. VII; *X*, lob. X. Line 1, "rostral level" of stimulation illustrated in Figs. 1 and 2; line 2, "caudal level" of stimulation, Fig. 3.

FIG. 2. An enlarged view (×58) of the area contained within the rectangle of Fig. 1. The acoustic commissure (*ac*) divides the *mlf* into supra- and infra-commissural bundles. Note that the large myelinated fibers of the *mlf* do not extend to the ventral midline of the medulla.

FIG. 3. View (×58) of the same area as Fig. 2 but corresponding to level 2 of the inset figure.

FIG. 4, 5 and 6 (×580). Fig. 4. Transverse section of the decussation of right and left Mauthner fibers. Note the relative thickness of myelin sheath and axon, lack of homogeneity in the sheath and dissymmetry in size of the two axons. Fig. 5 is a section 90μ caudal to that of Fig. 4; the axon of the Mauthner fiber indicated by the arrow has expanded and stains less deeply. Fig. 6 from same section as Fig. 5, illustrates a typical fiber area of *mlf*, *ic*.

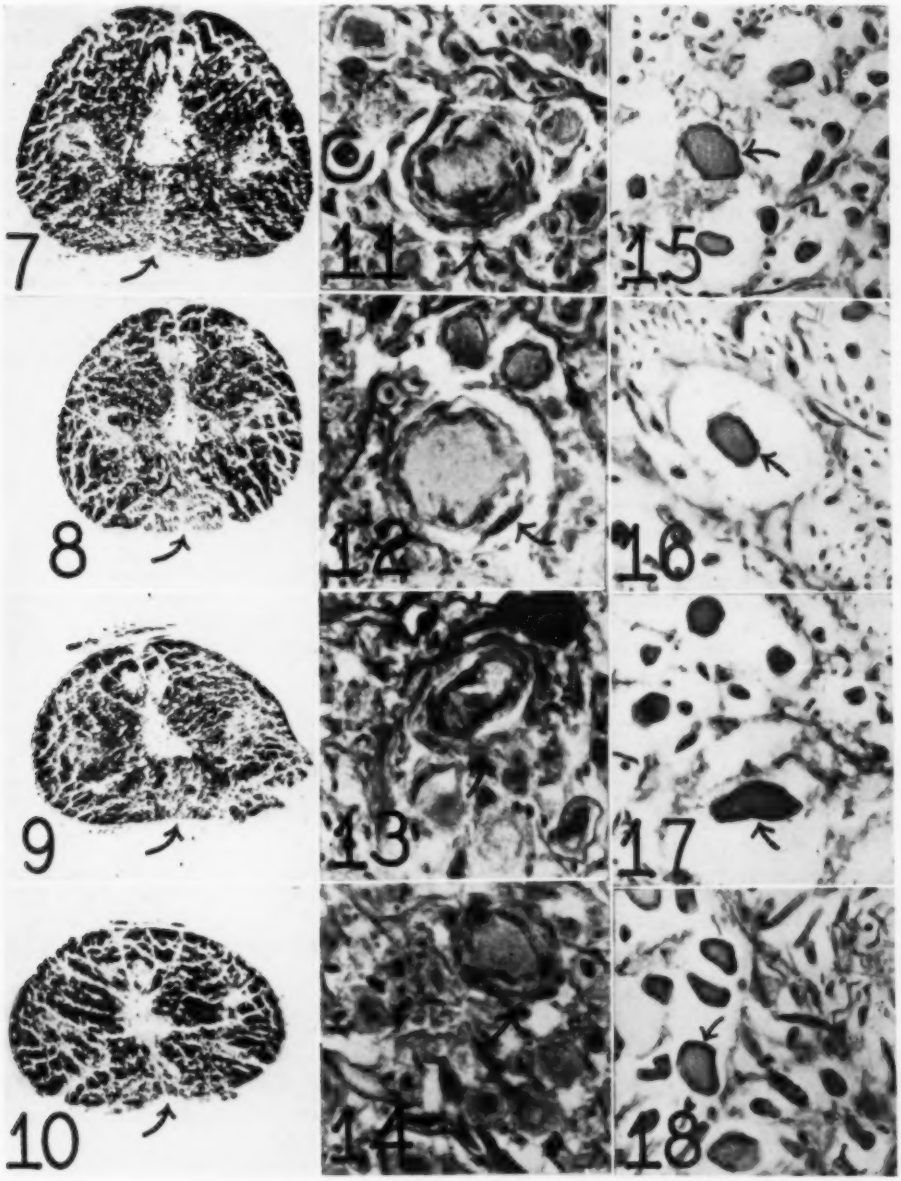

FIG. 7–10 (×35). Low-power orientation series of the spinal cord of *Ameiurus*, representing levels 60, 75, 90 and 115 mm. caudal to the origin of the Mauthner fibers. Cord length 140 mm. Arrows indicate the situation of the ventromedial fiber bundle. Formol bichromate fixation, Weigert mordanted, Kultschitzky stain.

FIG. 11–14 (×580) allow comparison of a single Mauthner axon and its sheath at cord levels comparable to those illustrated in Fig. 7–10. In each figure arrow ends at the outer margin of the myelin sheath. In Fig. 11 and 12 medium-sized Müller fibers (11μ) are included for comparison. Cord length 66 mm., formol chrome sublimate perfusion, Azan Mallory stain.

FIG. 15–18 (×580). Mauthner axon (arrows) in silver preparations; levels 9, 35, 71 and 115 mm. caudal to the origin of the Mauthner fiber. Note that at the 115 mm. level this Mauthner axon was no larger than an adjacent Müller axon. Cord length 140 mm.; "two-hour" protargol method.

Table 1. Diameters of Mauthner and Müller fibers. Average outside diameters in microns of two Mauthner and ten largest Müller fibers. Total length of cord about 140 mm. in all cases except the first two where it was 66 mm.; in these two cases the cord level measurements were corrected by the appropriate factor.

Control Series

Date and Method	Class of Fiber	Medulla	Cord level corresponding to conduction distance of:				
			30 mm.	60 mm.	75 mm.	90 mm.	115 mm.
11-1-39 W	Mauthner	32.0		43.0	30.0		26.0
	Müller	15.8		22.4	20.1		18.7
11-2-39 FSA	Mauthner			32.5		29.5	27.0
	Müller			17.3		16.0	13.5
6-8-40 W	Mauthner			38.0	34.0	29.0	28.5
	Müller			21.3	18.7	14.6	14.5
7-10-40 FSA	Mauthner	39.0					
	Müller						
7-10-40 FSA	Mauthner	33.0					
	Müller						
7-10-40 FSA	Mauthner	32.0					
	Müller						
7-10-40 FSA	Mauthner	40.0					
	Müller						
7-15-40 W	Mauthner			36.0		**	
	Müller			13.0		10.6	
7-15-40 W	Mauthner			33.0*		**	
	Müller			18.1		14.2	
7-15-40 W	Mauthner			28.0*			
	Müller			13.5			
7-15-40 W	Mauthner			29.5		28.5	
	Müller			13.9		11.1	

Experimental Series

5-8-40 W	Mauthner	39.5		41.0		22.0	
	Müller	16.3		16.3		12.8	
5-16-40 FO	Mauthner		38.0	38.0		28.0	45.0
	Müller		20.2	19.6		15.3	17.7
5-20-40 FO	Mauthner			30.2	**		
	Müller			16.1	12.7		
6-1-40 FO	Mauthner					**	
	Müller					19.4	

Table 1—Continued.
Experimental Series—Continued

Date and Method	Class of Fiber	Medulla	Cord level corresponding to conduction distance of:				
			30 mm.	60 mm.	75 mm.	90 mm.	115 mm.
6-25-40 FO	Mauthner		39.0	36.0		31.5	26.5
	Müller		16.7	17.6		15.7	11.8
7-8-40 O	Mauthner			33.5	26.0*		
	Müller			19.6	20.8		
7-9-40 O	Mauthner				29.5		26.0
	Müller				19.7		19.3
7-9-40 O	Mauthner			36.0		43.0	
	Müller			22.5		21.4	

FSA—fixation by perfusion of chilled formol chrome sublimate; processed by accepted cytological procedure; Azan Mallory stain.

W—fixation by immersion in formal bichromate mordanted in Weigert; stained by Kultschitzky's method.

O—fixation by immersion in 2 per cent osmic acid.

FO—fixation in aqueous 10 per cent formol; stained in the block by 2 per cent osmic acid.

* Only one Mauthner fiber identified.

** Neither Mauthner fiber identified.

In Fig. 2 the area enclosed in the rectangle of Fig. 1 has been enlarged. Examined in conjunction with Fig. 1 it illustrates that the densely packed large myelinated fibers of the *mlf* do not extend to the ventral midline of the medulla and that elsewhere than in the *mlf* and *llf* the myelinated fibers in the medulla are not disposed in large longitudinally coursing bundles. Figure 3 shows the *mlf* at the level (hereafter designated as the "caudal level") of line 2 of the inset figure. The relations of the *mlf* and *llf* have not changed significantly from the "rostral level," though the commissure is much diminished.

2. *Course, size and histological characteristics of Mauthner fibers at medullary and cord levels.* Following their decussation at the level of the acoustic commissure (Fig. 1 and 2) the two Mauthner fibers continue caudally in the superficial zone of the *mlf* (Fig. 3) and enter the ventromedial area of the spinal cord where they come to lie just ventral to the central gray (Fig. 7–10). Both Mauthner fibers could be identified as far as 90 mm. caudal to the level of the acoustic commissure in most of the fish, and even at 115 mm. it was frequently possible to distinguish them from the Müller fibers (see page 227) by their greater size and sheath thickness.

With appropriate care in the orientation of blocks of spinal cord for embedding, the outside sheath diameter of the same Mauthner fiber may be measured in the medulla and at various cord levels. In Table 1 the average diameters of the two Mauthner fibers are presented.

The members of each pair of the 6 measured at the medullary level were of nearly equal size, and varied between the limits of 32 and 40μ. Usually the Mauthner fibers are not significantly reduced in outside diameter for the first 60 mm. of their course through the spinal cord (140 mm. cord length); but farther down in the cord the size may be diminished so that a fiber measuring 40μ at 60 mm. may measure 25μ at 90 mm. (Table 1). Local fluctuations in diameter also occur, and these may be much accentuated in cords fixed in formalin and later osmicated. In the single Mauthner fiber selected for the photomicrographs of Fig. 11 to 14, the limits of chance fluctuation in fiber diameter as well as axon-sheath ratio are apparent by comparison of Fig. 11 and 12. Figures 13 and 14 at the same magnification show the reduced diameter which usually characterizes the caudal extremity of the Mauthner fiber.

The sheath of the Mauthner fiber reduces osmic acid, stains blue black by the Weigert method, and is soluble in fat solvents. In cytological preparations (formol chrome sublimate-Azan Mallory) the myelin sheath and axon color differentially red and blue respectively (Fig. 4 and 5 and 11–14). The red matrix of the myelin sheath is latticed by concentrically disposed slender blue fibrillae, which to our knowledge have not been previously described. Typical nodes of Ranvier were not observed, but node-like reductions in the diameters of the axons occur; these are accompanied by dense staining. This is evident by comparison of Fig. 4 and 5 which illustrate closely adjoining cross-sections of the same fiber. The axoplasm appears homogeneous save for scattered mitochondria; an axolemma was not distinguished and there was no separation into cortical and medullary zones (see also Fig. 15–18, "short protargol" method).

The axon as well as the outside diameter of each of the two Mauthner fibers has been measured in four medullas prepared cytologically. Since there was no retraction of the axon from the encasing sheath in any of these, the measurements can be considered accurate within the limits of shrinkage incurred during fixation. The eight fibers thus measured had an average axon diameter of 18.5μ, outside diameter of 37.2μ, the ratio of the former to the latter being 0.5. Four spinal levels of a single Mauthner fiber were similarly measured. These corresponded to the usual 60, 75, 90 and 115 mm. conduction distances used in the experimental series. The ratios of axon to outside diameters in μ were respectively

$$\frac{16.0}{27.5}, \quad \frac{24.0}{30.0}, \quad \frac{14.0}{18.5}, \quad \frac{13.0}{23.0}.$$

There was no evidence to suggest that these ratios were significantly different in smaller and larger fish (66 and 140 mm. total cord length).

3. *Myelinated fibers which enter spinal cord from median longitudinal fasciculus.* The giant Mauthner fibers can be traced individually through the spinal cord, but there is no direct anatomical evidence bearing upon the number of other large myelinated fibers which pass from the *mlf* into the ventromedial region of the cord. Such evidence could only be obtained by

Marchi degeneration following medullary lesions, or retrograde degeneration occurring subsequent to division of the spinal cord, and these procedures have not been carried out. However, since the continuity of the *mlf* with the ventromedial cord area is apparent in serial histological sections through the medullospinal junction, that area certainly should contain a high percentage of fibers derived from the *mlf*.

Figures 7 to 10 are low power photomicrographs of 5μ Weigert sections which illustrate the relative size of the cord and its contained gray matter at distances 60, 75, 90 and 115 mm. caudal to the level of the acoustic commissure. The ventromedial fiber bundle (arrows) is most apparent in Fig. 7 and 8 (60 and 75 mm. respectively). Here the close aggregation of thin sheathed fibers of large size has caused the area to stain less intensely than the remainder of the white matter. In Fig. 9 and 10 (90 and 115 mm. respectively) the larger myelinated fibers of the bundle, with the exception of the Mauthner fibers, have approached the ventral periphery of the cord. The zone immediately surrounding the Mauthner fibers, which lies beneath the central gray, is made up chiefly of smaller myelinated and non-myelinated axons. For convenience we shall refer to the large thin-sheathed medullospinal axons of the ventromedial bundle as Müller fibers.

The sheaths of the Müller fibers are similar in staining properties and solubility to those of the Mauthner fibers, but they do not exhibit evidence of concentric lamination of the myelin matrix. Figure 6 is a cross-section of a group of the fibers of the *mlf*, *ic* bundle near the "rostral level." The Müller fibers at this level are scattered among numerous smaller ones so that no fair comparison may be made between average size in the *mlf* and in the ventromedial bundle of the spinal cord.

The measurements of the Müller fibers at different cord levels have been abridged to the average outside diameters of the 10 largest fibers (Table 1). In measurements of the diameters of at least 100 fibers selected at random from the ventromedial bundle at each cord level recorded in the table, the fibers were found to grade in size from the high value published to a minimum of 2 to 4μ; non-myelinated fibers also occur. From Table 1 it is evident that the Müller fibers are somewhat reduced in diameter between the 60 and 115 mm. levels. In one specimen (6/25/40) an average reduction of 5.8μ occurred, in another (5/16/40) the average reduction in diameter was only 1.9μ.

In four cord levels of one preparation, measurements in μ of both the axon and the outside diameters of the 10 largest Müller fibers were averaged. These levels correspond to the usual conduction distances of 60, 75, 90 and 115 mm., and the ratios are directly comparable to those given for the single Mauthner fiber measured in the same sections (page 230). The ratios for the Müller fibers were respectively $\frac{15.2}{19.6}$, $\frac{14.0}{19.2}$, $\frac{12.2}{16.6}$, $\frac{12.0}{17.2}$ at the 4 levels.

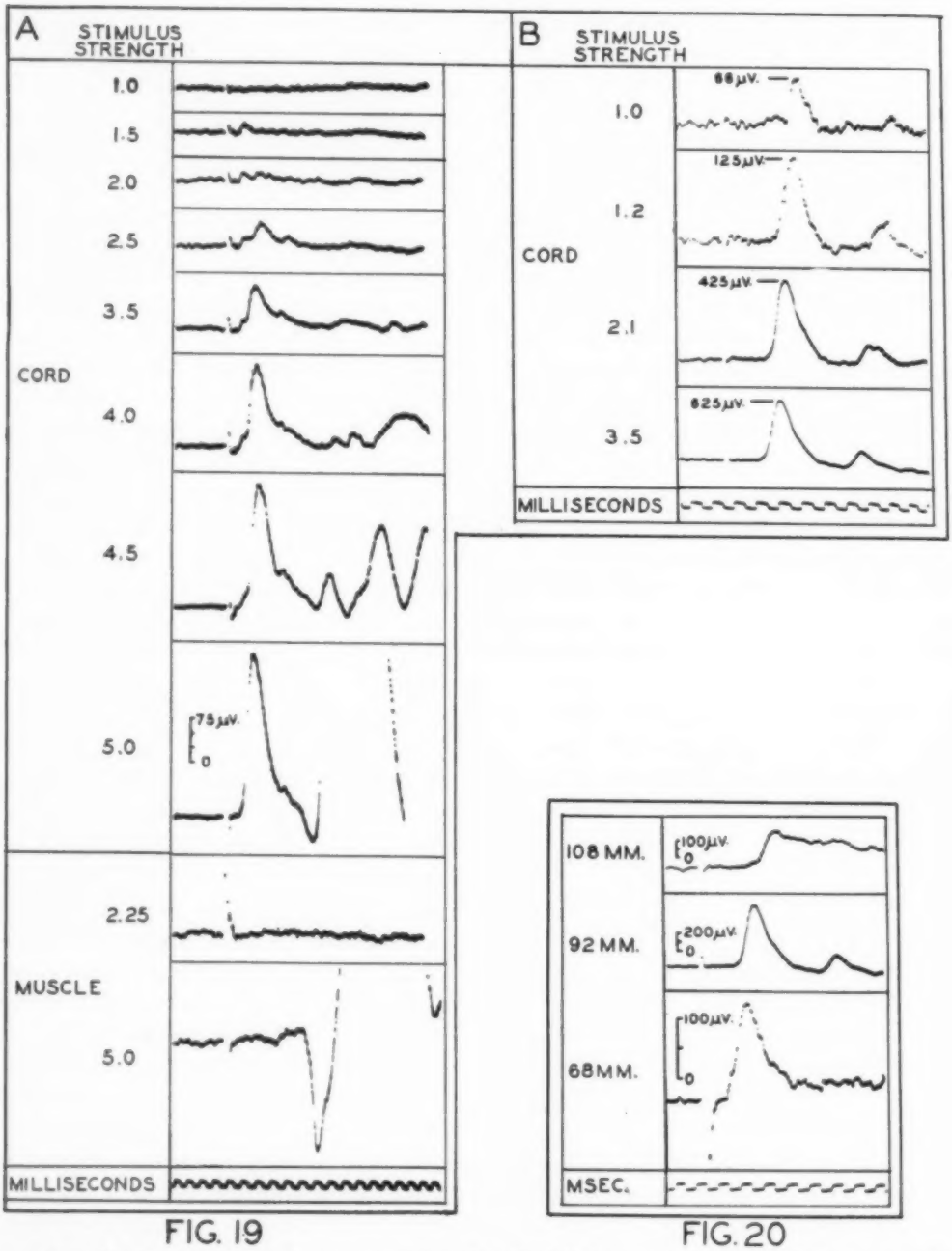

FIG. 19

FIG. 20

FIG. 19. Cord and muscle responses to stimuli of different strengths. Relative strength of stimulation indicated (weakest used in each case = 1).

A. Pair of stimulating electrodes 0.5 mm. behind "rostral level." First 8 records: two recording electrodes in cord, more rostral one 52 mm. from stimulating electrodes. Last 2 records: recording electrodes on muscle, 5 mm. from position of electrodes in cord. All records of series at same amplification. Temperature 13.5°C. 4/18/40.

B. Pair of stimulating electrodes 0.5 mm. in front of "caudal level." Single recording electrode in cord, 92 mm. from stimulating electrodes. Amplification decreased for records with greatest strengths of stimulation. Temperature, 12.5°C. 6/3/40.

FIG. 20. Responses at 3 conduction distances. Same preparation as for Fig. 19B; stimulus maximal. Conduction rate, 50–56 m./sec.

Experimental Observations

1. *Continuous activity in cord.* Early in some experiments, the cord showed rhythmic activity consisting of waves of several hundred microvolts 0.6–1.4 sec. apart. When present, this activity was at times increased in amplitude though not in frequency by stimulation of the saccular otolith, or occasionally it was induced by such stimulation. It seems to be similar to the rhythmic activity observed by Shurrager (16) in brains recently isolated from catfish, and to like potentials recorded from the central nervous systems of many animals by other workers. In addition the catfish cords showed a persistent, continuous activity consisting of irregular oscillations of a few microvolts, clearly visible in all records taken with sufficient amplification. This background activity interfered only with the smallest waves induced by stimulation, and no effort was made to control it.

2. *Form of response in cord; conduction time.* Electrical stimulation of the medulla results in the appearance in the cord of an electrical disturbance which is recorded as a complex of waves, chiefly negative in sign. If the stimulating electrodes are inserted vertically at the "rostral level" so that they are in the region of the Mauthner-Müller cells, or if they are inserted behind this level, the response will usually appear in the cord within several msec., and the exact time interval will be such that the rate of the fastest impulses (calculated from conduction time and distance without allowing for the latent period) is 40–60 m./sec. The conduction time between these medullary levels and the cord is the same whether the impulse is transmitted away from or towards the medulla. It may be concluded that below the acoustic commissure there are no synapses in the pathway of the fast impulses. If the stimulating electrodes are placed rostral to the commissure, the conduction time will be significantly (1 msec. or more) longer than at lower levels; evidently synapses in the pathway of the fast impulses occur immediately in front of the "rostral level," but the situation has not yet been investigated in detail electrically.

The cord response to maximal medullary stimulation at or behind the "rostral level" (Fig. 19 and 20) consists mainly of a relatively large single negative wave lasting 1–3 msec. (maximum amplitude typically 100–500μV.). This main wave may be preceded by a much smaller wave of 5–20μV. lasting 0.5 msec. or more, and may be followed by other small waves for an interval of 5 msec. or more. The conduction rate for the main wave varies from the figure given above for the fastest impulses (40–60 m./sec.) to 10–20 m./sec. for the slowest ones, while the rate for the small initial wave when present is never less than 50 m./sec. and never significantly greater than the fastest rates observed for the main wave in the absence of the initial wave.

The fastest conduction rate as determined at various distances tends to be the same in each preparation (Fig. 20), and the recorded form is not markedly affected by temporal dispersion except sometimes at conduction distances greater than 100 mm. (cf. Fig. 20, records at 92 and 108 mm.;

Fig. 22). Finely-measured changes in depth of the recording electrode at any one level in the cord alter only the magnitude of the response, not its time relations or recorded form. The form can however be modified by changing the strength of stimulation or the locus of stimulation in the medulla.

The effect of strength of stimulation on the recorded form is illustrated in Fig. 19 and 21. The preparation used for Fig. 19A contained a few fast fibers (62 m./sec.) which produced a small wave with low strengths of stimulation. A later wave, formed by the response of the numerous fibers with conduction rates of 35 down to perhaps 20 m./sec., came in gradually with stronger stimulation, while with strong enough stimulation, the potential was recorded as a single continuous wave. The preparation used for Fig. 19B gave no evidence of fibers with faster rates than about 50 m./sec., and a doubtful initial wave with weak stimulation only.

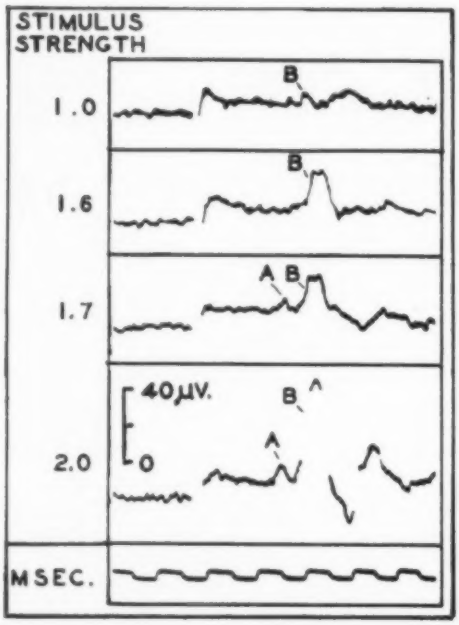

FIG. 21. Single fiber responses. Monopolar stimulation at "rostral level." Relative strength of stimulation indicated. One recording electrode in cord, 96 mm. from stimulating electrodes. Conduction rates: wave A, 60 m./sec.; wave B, 48 m./sec.; start of slower wave in first record, 37 m./sec. Temperature 15.5°C. 7/9/40.

With near-threshold strengths of stimulation it was usually possible, especially if the stimulus was applied at the "rostral level" (see below), to obtain records consisting of a succession of small, discrete waves, indicating groups of fibers with different conduction rates more or less equally accessible to the stimulus. With stimulation sufficiently near threshold, some of these small waves have the characteristics of single fiber responses, though more of them probably represent the activity of homogeneous groups of very few fibers. A change of less than 1 per cent in stimulus strength may suffice to bring in or leave out one of these groups; that they actually are groups and not single fibers is indicated by the longer duration and higher voltage of their responses than of the true single fiber responses recorded, and also by their splitting up into smaller waves with rapidly repeated stimulation (not illustrated). Apparently the fibers composing the groups differ more in their recovery characteristics than in other properties.

In the records reproduced in Fig. 21, the lowest strength of stimulation produces a small, discrete wave (B) interpreted as indicating the activity of

a single fiber with a conduction rate of 48 m./sec., and a longer later wave which probably results from the activity of several fibers with slightly different conduction rates in the neighborhood of 35 m./sec. An increase of 63 per cent in stimulus strength does not increase the potential recorded from these slow fibers, but more than doubles the height of wave B. The cord evidently contained a number of excitable fibers conducting at about 48 m./sec. A further slight increase (4 per cent) in the stimulus makes no change in wave B, but introduces a preceding small wave (A, conduction rate 60 m./sec.), the original height of which is not more than doubled by further increase in stimulus strength.

Waves A and B resemble both in magnitude and duration the smallest of the initial waves described above as preceding the large main response. It may be concluded that such waves at their smallest result from the activity of single fibers, but because they do not sufficiently exceed in size the waves of continuous background activity, it cannot be concluded that they accurately reproduce the true form of the individual spike. Definite statements regarding this form must await more selective recording of the potential; the apparent duration (0.4–0.5 msec.) in the available records is probably too short. However, it is worth noting that the form seems to be the same for all the single fibers observed, with conduction rates from 60 down to 15 m./sec.

The importance of the position of the stimulating electrodes in the medulla is illustrated in Fig. 22. The records of series I were obtained from the caudal portion of the cord following stimulation of the medulla at the "rostral level," those of series II from the same position in the cord following medullary stimulation at the "caudal level." Comparison of the two series as wholes brings out the greater tendency to separation of the waves and the lower maximum potential (about half that in series II) with stimulation at the "rostral level" (cf. the discrete potential waves and the similar stimulating position of Fig. 21). In each series, the response of the fast fibers is very small at the most dorsal effective stimulating depth, but increases rapidly with increasing depth of the electrodes; within the limits of error of location of the stimulating electrodes, the maximum responses in each series is obtained with stimulation in the region of the *mlf*. Stimulation is however still effective, particularly on the slower fibers at the "rostral level," when it is applied well below the *mlf*, even in the fluid below the medulla.

3. *Effect of temperature on conduction rate.* The results described above were obtained at temperatures varying from 10° to 15°C. for the whole group of experiments, but constant within about a degree for any one experiment. In a few experiments, records were made at several distinct temperatures; data obtained on the conduction rate of the fastest fibers in these experiments are summarized in Table 2. The temperature coefficient (Q_{10}) calculated from these data varies from 1.3 to 1.9 for temperatures ranging from 23° to 8°C., the higher values falling in the lower temperature ranges.

4. *Recovery cycle.* Observations upon the recovery cycle of the fast fibers

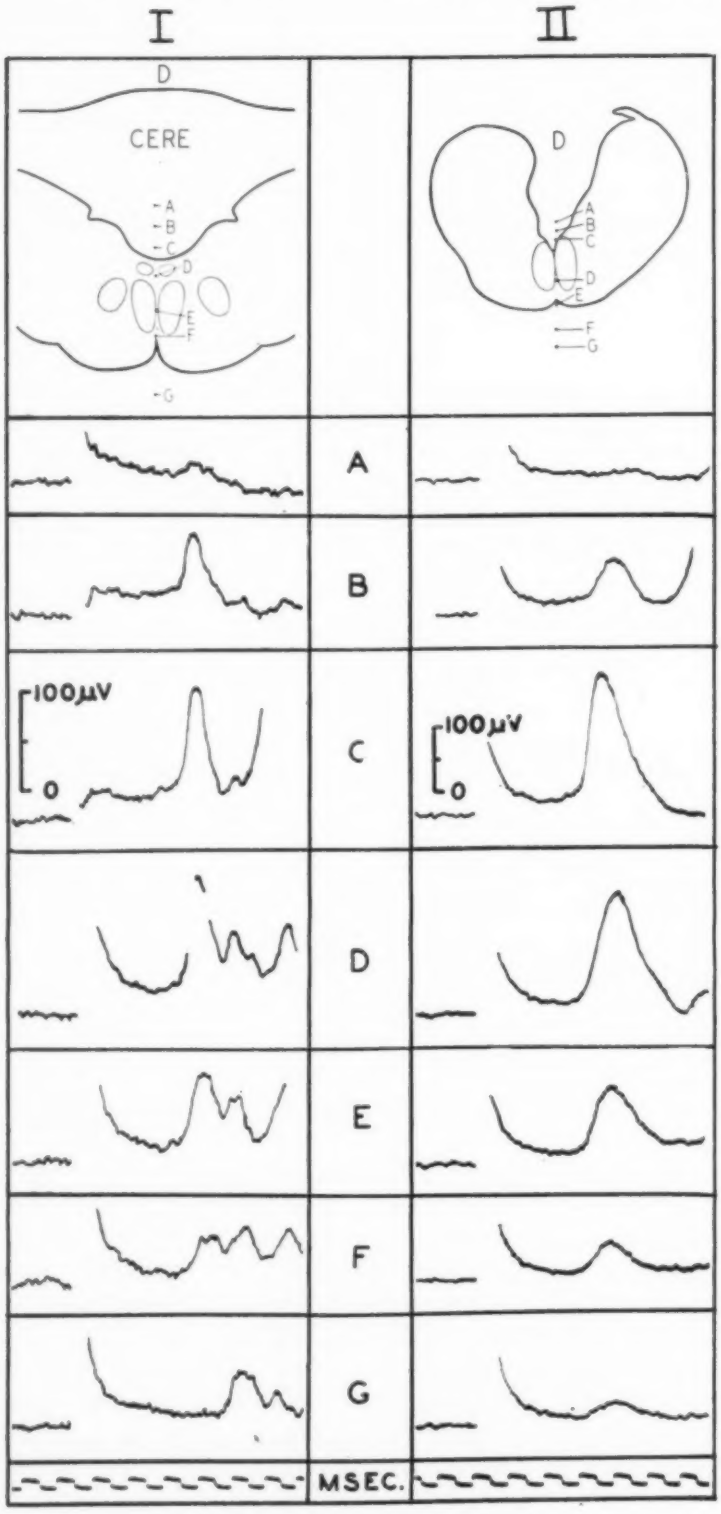

FIG. 22. *See next page for legend.*

in the cord following stimulation in the medulla are interfered with by the presence of slow pathways, activated by shocks of the strength required for the observations in question, and apparently more readily activated by two successive shocks than by a single one. Nevertheless, sufficiently accurate data have been obtained with medullary stimulation through a single pair of

Table 2. Temperature coefficient of conduction rate. Stimulating electrodes in medulla between "rostral" and "caudal levels"; recording electrodes on cord.

Date	Temperature °C.	Conduction distance mm.	Conduction rate m./sec.	Temp. coef. Q_{10}
2/7/40	16	58	58	
				1.4
	23		73	
				1.3
	15		58	
				1.5
	11.5		48	
				1.9
	8		39	
4/20/40	13.5	67	42	
				1.3
	21.5		51	
5/9/40	11.5	61	35	
				1.9
	17.5		51	
				1.5
	22		61	

electrodes for conditioning and testing shocks to justify the following general statements about the recovery cycle of the fast fibers. The absolutely refractory period is of the order of 1 msec., while the relatively refractory period varies from 2 to 10 msec. During the relatively refractory period, the rate of conduction is slowed, as in peripheral nerve. This period is usually followed by a period of supernormal excitability which lasts several hundred milliseconds; the maximum excitability (greatest observed, 10 per cent above the resting excitability) falls 10–20 msec. after the conditioning shock. The

FIG. 22. Effect of changing position of stimulating electrodes. Pair of paramedian stimulating electrodes, 2 mm. apart; depth of insertion into medulla measured micrometrically. Recording microelectrode on cord, 110–115 mm. below stimulating electrodes. Amplification same in all records of each series; greater in I than in II, as indicated by calibrations in records C. Temperature, 10.5°C. 6/25/40.

I. Stimulating electrodes at "rostral level"; the lettered dots in drawing I indicate the approximate positions of the electrodes when stimulation produced the records similarly lettered in series I. The ovoid areas next to the midline in the drawing include the area enclosed within the rectangle of Fig. 1.

II. Stimulating electrodes 0.5 mm. in front of "caudal level"; lettered dots of drawing II and series II records related as in I.

existence of a subnormal period following the supernormal period has not been observed following a single shock, but considerable subnormality is developed by repetitive stimulation, even at low frequencies.

Discussion

The above description of the properties of catfish central fibers clearly shows their close resemblance to amphibian peripheral fibers of the A group. The catfish fibers are larger than frog A fibers in general, but the outside diameter of the Müller fibers is not significantly greater than the 20μ reported for some bullfrog A fibers by Erlanger and Gasser (9). The axon diameter of these two sets of fibers is probably also about the same: if the value 0.75 holds for the ratio $\frac{\text{axon diameter}}{\text{outside diameter}}$ of the bullfrog fibers as of large A fibers of the green frog (Schmitt and Bear, 15), the axon diameter of the large bullfrog fibers is about 15μ, actually greater than the measured axon diameters of the Müller fibers (average value, 13.4μ). The Mauthner fibers are of course somewhat larger, even in the cord and even with respect to axon diameter; in the medulla and with respect to outside diameter they are notably larger than the frog fibers.

In estimating the resemblance in physiological properties, allowance must be made for the (roughly) ten-degree difference in temperature between most of the experiments on catfish and those on frogs. At 20–25°C., frog fibers conduct up to 55 m./sec. (Erlanger and Gasser, 9), their absolutely refractory period is as short as 0.8 msec. (Bishop and Heinbecker, 4), their relatively refractory period 1.5 msec. or longer (Graham, 12). When appropriate allowance is made for temperature, the catfish fibers seem actually to conduct more rapidly than frog A fibers—a state of affairs consonant with their larger size—and to recover no less rapidly.

Other points of similarity between the two sets of fibers are the marked supernormal phase of the recovery cycle and the temperature coefficient of the conduction rates. The temperature coefficient is similar in magnitude as well as in being greater in lower temperature ranges (Gasser, 10). The only possible significant difference between the two sets of fibers is in the time-relations of the spike, since the spike rising time of frog A fibers is not less than 0.3 msec. (Blair and Erlanger, 5) at room temperature, while the apparent duration of the entire spike of the catfish fibers even at the lower temperature of these experiments does not greatly exceed this time interval. But the uncertainty involved in this observation on fibers in tissue with continuous background activity precludes attributing significance to this difference between the two sets of fibers in view of their similarity in all other respects.

The source of the responses from the catfish cord is believed to be the larger fibers of the ventromedial bundle for the following reasons. (i) The response is maximal when the stimulating electrodes are placed in the region of the *mlf* in the medulla (Fig. 22), from which the ventromedial fibers of the

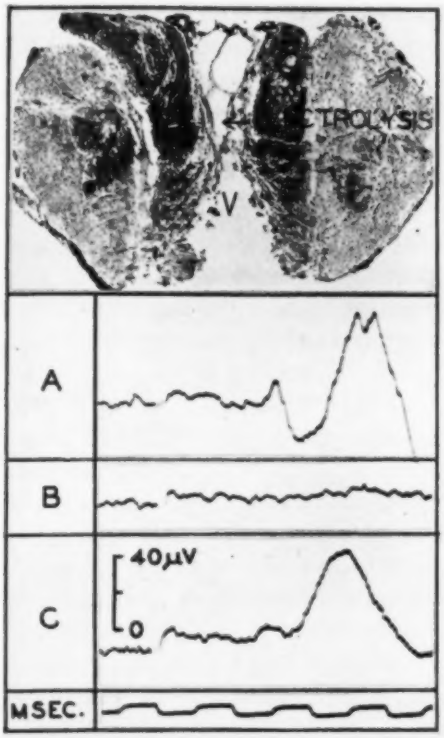

FIG. 23

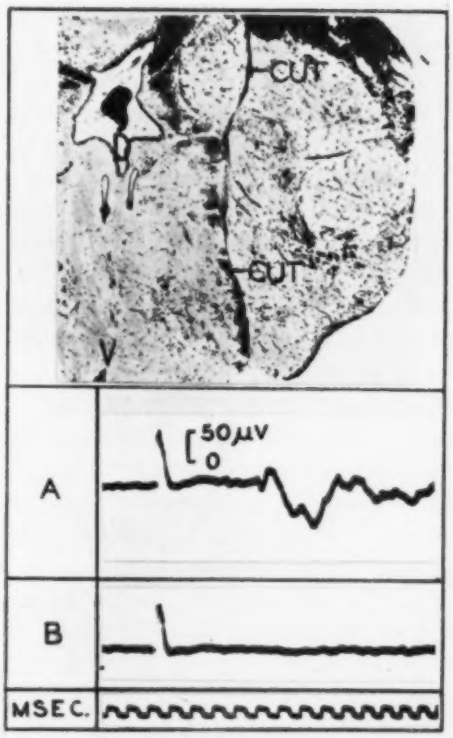

FIG. 24

FIG. 23. Effect of electrolytic destruction of midline of medulla. Stimulating electrodes in cord, 94 mm. from first position of recording electrode. Recording micro electrode in medulla; first position, "caudal level," records A and B; second position 4 mm. behind "caudal level," record C. After record A taken, electrolysis with 15 mA. for 30 seconds at first position; records B and C then taken. The response is completely eliminated at the region electrolyzed (record B), but still present in the ventromedial fibers caudal to this region (record C). Photomicrograph: cross-section (colored with haematoxylin and eosin) near caudal end of lesion shows its dorso-ventral and lateral extent; rostro-caudal extent of lesion 4 mm.; no visible evidence of lesion at second position. V = ventral midline of medulla. Temperature, 10°C. 7/1/40.

FIG. 24. Cord response to stimulation of the saccule before (record A) and after (record B) separation of the incoming paths of the ipsilateral VIIIth nerve from the *mlf*. Pair of stimulating electrodes placed in contact with saccular otolith; recording electrodes in cord 70 mm. away. Photomicrograph: cross-section (colored with cresyl violet) of ipsilateral half of medulla showing cut at "rostral level," about 2 mm. in rostro-caudal length, through the acoustic commissure and juxtaposed neuropil. Temperature, 13.5°C. V = ventral midline of medulla. 2/9/40.

cord come; when the stimulating electrodes are moved laterally in the medulla the response disappears unless the strength of stimulation is increased. (ii) The response to stimulation at the "caudal level" is greater and less scattered but no faster than the response to stimulation at the "rostral level" (Fig. 22). This is in agreement with the histological relationships, since those fibers of the ventromedial bundle that arise from cells lying behind the "rostral level" would be stimulated from electrodes at the "caudal level"

but not at the "rostral level"; these fibers do not differ in size (therefore presumably not in conduction rate) from those arising more rostrally. (iii) Stimulation of the cord produces responses recorded from the medulla with the same conduction rate as impulses propagated in the reverse direction, and these responses can be eliminated by electrolysis of the *mlf* (see Fig. 23 and legend). (iv) Cord responses which are believed to occur in the same fibers as those from medullary stimulation can be produced by stimulation of the saccule; their time of appearance in the cord is of course later by the period of at least one synaptic delay. Description of these responses is not included in this report, but Fig. 24 gives an examples of them, and the effect of severing the endings of the nervous pathways from the saccule to the *mlf*. This experiment leaves no room for doubt that the cord responses to saccular stimulation occur in the fibers of the *mlf*. (v) The conduction rates of the responses to medullary stimulation indicate that they must occur in large fibers, and most of the large fibers of the cord are in the ventromedial bundle. Moreover, the similarity of the ventromedial fibers to frog fibers in size, and of the catfish cord responses to frog A responses, indicates the correctness of this view.

While histological study of the individual cords from which records were made has not explained on the basis of fiber size the variations from experiment to experiment of either the form as recorded or the fastest conduction rate observed, the occurrence in many experiments of a small initial wave appearing with relatively weak stimulation and not increasing greatly in size with stronger stimulation, indicates the presence in the cord of a couple of fibers set off from a much more numerous group by their conduction rate. The conduction rate of the start of the main wave following such a small initial wave is roughly 0.8 of the rate of the small wave (see the typical relationship in Fig. 21). This ratio is much greater than the ratio between the average outside diameters of the largest Müller fibers and of the Mauthner fibers (cf. Table 1) but is about the same as the ratio between the average axon diameters of these fibers (0.75–0.8 as calculated from the data on pages 229 and 230). Therefore, on the hypothesis of direct proportionality between axon diameter and conduction rate (Gasser and Grundfest, 11), the assignment of the initial and main waves to the Mauthner and Müller fibers respectively seems warranted.

Summary

1. The spike potentials which appear in the spinal cord following electrical stimulation of the *Ameiurus* medulla resemble amphibian A fiber responses in conduction rate, recovery cycle, and temperature coefficient of conduction rate.

2. Since the fastest of these potentials travel at the rate of 50–60 m./sec. at 10–15°C., the fibers producing them must be larger than frog A fibers, if the usual theory relating fiber size and velocity applies.

3. Fibers of sufficient size to account for the cord spike potentials pass

into the ventromedial bundle of the spinal cord from the median longitudinal fasciculus of the medulla, which contains the axons of the Mauthner and Müller cells.

4. The outside diameters of the Mauthner fibers vary between 43 and 22μ in the cord and medulla, while the axon diameters vary between 24 and 13μ. The heavy myelin sheath accounts for about half of the total fiber diameter in the medulla; it becomes progressively thinner down the cord, without however becoming as thin in relation to fiber size as the sheaths of the other large fibers. The matrix of the Mauthner fiber sheath colors red in Azan Mallory, and is latticed by concentrically disposed, blue-staining fibrils.

5. The average outside diameters of the ten next largest fibers (Müller fibers) vary between 22.5 and 11.1μ at different levels and in different preparations. The average axon diameter is about 0.7 of this. There is no constant difference in relative sheath thickness at different levels, and the sheaths appear homogeneous.

6. In correlation with the diameter measurements of the Mauthner and large Müller fibers, the properties of the fast cord spike potentials indicate that they arise in these fibers. The effects on the response of changing the location of the stimulus in the medulla and the absence of other elements in the cord likely to produce such a response, point to the same conclusion.

7. Stimulation of the saccular otolith causes responses in the cord which apparently occur in the same fibers as the responses to medullary stimulation; the responses to saccular stimulation have been shown to pass through the median longitudinal fasciculus.

8. Responses of single fibers, of various conduction rates from 60 to 15 m./sec., may be recorded from microelectrodes in the cord, particularly if the stimulus is placed rostrally in the medulla. The single fiber responses all appear to have the same spike form.

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AVAILABILITY OF LACTIC ACID FOR BRAIN OXIDATIONS*

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THE FOOD requirements of the brain appear to be more limited than those of other organs. Whereas most parts of the body oxidize a mixture of both fat and carbohydrate, it has been repeatedly demonstrated that the R.Q. of both animal (1) and human (2) brain tissue is close to unity. There are three foodstuffs normally present in the body which yield an R.Q. of 1.0 on oxidation, namely, acetoacetic acid, glucose, and lactic acid. Goldfarb and Himwich (3) have shown that even with the high blood acetone levels of diabetic dogs the brain, in contrast to other organs, did not remove acetone substances from the blood. This indicates that acetoacetic acid, at least in the diabetic, does not serve as a substrate for brain oxidation.

All workers are agreed that brain oxidizes glucose, but the evidence for the oxidation of lactic acid is at present contradictory. *In vivo* studies reveal the removal of relatively small amounts of lactic acid from the blood traversing the brain whenever the level of blood lactic acid is raised (1, 4). However, under such conditions it is impossible to decide whether the lactic acid absorption is due merely to diffusion into the brain because of the higher concentration in the blood, or whether lactic acid is also actually metabolized by that organ. The second possibility is rendered doubtful by observations made on human subjects with basal values for lactic acid. Under these circumstances no absorption of lactic acid was revealed even during hypoglycemia when the brain is deprived of glucose (5). Mann and Magath (6) observed that lactic acid injected into hepatectomized dogs did not alleviate hypoglycemic convulsions, while glucose readily relieved the seizures. Studies of brain potentials in hypoglycemia have contributed valuable evidence on various aspects of brain oxidations. Hoagland, Rubin, and Cameron (7) showed that there are characteristic changes of the cerebral potentials of the cortex during insulin hypoglycemia. These changes are associated with a diminished oxygen uptake of the brain (8), occur despite the presence of a moderate lactacidemia and are reversed by glucose administration. There were similar variations in brain potential in dogs during hypoglycemia produced by evisceration and hepatectomy (Maddock, *et al.*, 9). These authors found that the electroencephalograms returned to normal after the administration of glucose, mannose, and maltose; but were unchanged following the

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administration of fructose, galactose, sodium succinate, sodium fumarate, sodium glutamate, sodium pyruvate, sodium hexose diphosphate, glyceric aldehyde, or a mixture of glyceric aldehyde and sodium glutamate. These results are contrary to those obtained *in vitro* by Warburg, Posener, and Negelein (10) and others, who found that the oxygen uptake of excised brain tissue is accelerated when either lactic acid or glucose is supplied as substrate.

In attempting to ascertain the function of lactic acid, one is met by the difficulty that the liver converts this substance to glycogen (11) which may then be broken down to glucose and released to the blood stream in that form. It would seem that crucial experiments to determine the utilization of lactic acid by the brain *in vivo* should include at least four factors: (i) increase of the level of lactic acid in the blood, either induced endogenously in the organism, or produced by the administration of that substance; (ii) absence of other substrates, as in hypoglycemic coma; (iii) evidence of utilization of lactic acid during hypoglycemia as indicated by increased utilization of oxygen by the brain, and clinical revival of brain function with recovery from coma; and (iv) elimination of the possibility of conversion to glucose by removal of the liver, or estimation of this possible source of error by actual determination of the glucose and lactic acid uptake by the brain.

METHOD

The metabolism of the brain *in vivo* was studied in both man and animals. The cerebral metabolism of the human subjects was estimated from analyses of the arterial blood entering the brain and the venous blood leaving the brain through the interval jugular vein. The arterial blood was obtained from either the brachial or femoral artery. The venous sample was taken from the internal jugular vein using the technique described by Myerson

Table 1. Effect of 20 g. of sodium lactate intravenously on cerebral metabolism of schizophrenic patients in insulin coma.

Control before lactate

Exp. no.	Vol. per cent oxygen			Mg. per cent glucose		Mg. per cent lactate		Circ. time
	Art.	Ven.	Diff.	Art.	Ven.	Art.	Ven.	
1	18.5	13.6	4.9	43	52			12
2	18.6	16.8	1.8			26		8
3	18.7	17.9	0.8	47	45	38	32	14
4	15.8	13.9	1.9	40	42	40	35	11
5	17.3	16.1	1.2	35		29	17	9
6	18.7	16.4	2.3	39	36			11
7	21.5	18.3	3.2	56	42	25	36	8
8	20.7	17.0	3.7	55	48	32	19	9
9	20.2	14.5	5.7					13
10	18.5	16.4	2.1			45	34	10
11	20.2	19.9	0.3					9
12	22.6	17.2	5.4			37	36	10
13	18.8	18.1	0.7	41	26	18	4	16
14	19.9	16.0	3.9	35	25	28	10	8
			2.71	43	40	32	25	10.6

From 5 to 20 min. after lactate

Exp. no.	Vol. per cent oxygen			Mg. per cent glucose		Mg. per cent lactate		Circ. time	Min. after
	Art.	Ven.	Diff.	Art.	Ven.	Art.	Ven.		
1	16.5	13.6	2.9	42	39			10	6
3	17.3	13.4	3.9	33	27	79		12	5
4	17.1	14.1	3.0	41	49	96	59	12	7
7	20.2	13.9	6.3	50	23	60	62	8	10
8	19.9	15.0	4.9	45	38	54	40	11	18
9	18.4	13.9	4.5					13	8
11	19.5	15.4	4.1					9	15
12	19.6	15.2	4.4			94	77	10	7
13	17.8	14.5	3.3	35	32	70	51	9	10
14	17.4	14.6	2.8	28	13	72	78		8
			4.01	39	32	75	61	10.4	

From 25 to 50 minutes after lactate

Exp. no.	Vol. per cent oxygen			Mg. per cent glucose		Mg. per cent lactate		Circ. time	Min. after
	Art.	Ven.	Diff.	Art.	Ven.	Art.	Ven.		
1	16.4	13.3	3.1	40	38			8	25
	17.3	11.4	5.9	40	48			9	45
2	19.1	14.9	4.2	58	55	42	44	8	25
	21.8	15.1	6.7	59	52	21	26	8	50
3	18.5	14.1	4.4	33	17	70	55	10	25
4	17.0	12.8	4.2	39	44	54	54	9	30
5	18.6	13.7	4.9	28		16	41	7	25
6	17.7	12.4	5.3	56	43			8	23
7	20.6	15.2	5.4	40	16	48	44	10	45
8	19.3	15.0	4.3	45		50		11	30
10	18.6	13.5	5.1			84		10	33
12	20.5	14.3	6.2			53		11	27
14	17.4	13.8	3.6	27	16	32	27		32
			4.87	42	37	47	42	9.1	

Halloran and Hirsch (12). The blood samples were analyzed for carbon dioxide, oxygen (13), lactic acid (14), and glucose (15). The velocity of the blood flow through the peripheral circulation was measured with the cyanide method of Robb and Weiss (16). The availability of the lactic acid was estimated in human subjects while the patients were in deep insulin coma, and again at various intervals after administration of 20 g. of r-sodium lactate in 14 experiments, according to a method previously described (17).

In the animal experiments, dogs were hepatectomized under ether anesthesia, and were studied after they had completely recovered from the anesthetic. The liver was extirpated by the one stage method of Markowitz, Yater, and Burrows (18). The intestines, spleen and pancreas were simultaneously removed. In the various experiments glucose and lactic acid were injected intravenously. Blood samples were analyzed for glucose (19), lactic acid, and oxygen.

Results

The experimental data on human subjects are presented in Table 1. The average oxygen uptake during coma, before the administration of lactate,

was 2.71 vol. per cent, the glucose uptake was 3 mg. per cent, and the lactate uptake was 7 mg. per cent. Observations made within the first 20 min. after intravenous injection of 20 g. of racemic sodium lactate revealed a moderate increase in the oxygen and glucose uptake, and a large increase in lactic acid uptake. Studies made from 25 to 50 min. after injection revealed a slightly larger oxygen uptake, and a return of the lactate uptake toward the earlier value despite the high blood lactic acid levels persisting at this time.

Table 2. Changes in glucose and lactic acid in hepatectomized dogs.

	Sugar		Lactic acid		
	Art.	Ven.	Art.	Ven.	
Dog No. 1					
45 min. postoperative	55	47	82	81	
2 hours postoperative		18		96	Convulsions
2 hours 10 min. postoperative	138	141	110	104	8 min. after 4 g. glucose recovery
Dog No. 4					
55 min. postoperative	70	65	67		
2 hours 5 min. postoperative	34	26	98	101	Convulsions
Dog No. 6					
1 hour 20 min. postoperative	25	19	78		Convulsions
1 hour 40 min. postoperative	250	195	135		8 min. after 6 g. glucose recovery
3 hours 12 min. postoperative	27	17	162	163	53 min. after 8 g. sodium lactate

The clinical condition of only two patients showed any change. In experiment 2 there was a definite diminution of the depth of coma, while the patient in experiment 7 aroused sufficiently to answer questions. The latter was fairly well oriented, but after 20 min. again lapsed into coma. This patient habitually roused from coma after any manipulations. In both these cases the arteriovenous oxygen differences approached the normal during the period of arousal. The remaining patients showed no clinical change. The cyanide circulation time showed no significant change.*

The results of the animal experiments are presented in Table 2. Seven dogs were studied. Dogs No. 1, 2, and 3 had high levels of lactic acid as a result of the ether anesthesia, which further increased after the hypoglycemic convulsions. Despite the lactacidemia, convulsions occurred as hypoglycemia developed. Furthermore, the convulsions were immediately relieved by the injection of glucose. When glucose injections were withheld from dogs No.

* Similar experiments subsequently performed, with brain blood flow simultaneously recorded with a modification of the Gibbs thermostromat revealed no significant changes in brain blood flow in the course of the experiments (see HIMWICH, H. E., BOWMAN, K. M., DALY, C., FAZEKAS, J. F., WORTIS, J., and GOLDFARB, W., Changes in cerebral blood flow and arteriovenous oxygen difference during insulin hypoglycemia. *Proc. Soc. exp. Biol. N. Y.*, 1940, *45:* 468–469.)

4 and 5, the high level of lactic acid did not save the animals. Dog 6, which recovered from hypoglycemic convulsions after intravenous glucose administration, succumbed later to hypoglycemia despite the intravenous injections of sodium lactate. A similar observation was made with dog 7.

Discussion

Estimations of brain metabolism by oxygen, glucose and lactic acid differences in the arterial and venous blood are subject to two sources of error, (i) diffusion of substances between tissue and blood due merely to changes in concentration, and (ii) changes in blood flow through the brain. The first of these factors could not be measured, but since the determinations were made at varying intervals after the administration of lactate, it seems probable that the factor of diffusion would have resulted in variable results in both directions.

The second possible source of error involved in this method of studying brain metabolism has been recently emphasized by Abramson *et al.* (20), who believed that the diminished oxygen uptake observed in insulin shock may be attributable to changes in blood flow. These authors noted an increased blood flow in the extremities of 6 of 11 patients receiving insulin therapy for schizophrenia. Despite the fact that brain blood flow was not recorded, they concluded that their evidence indicated that there might well be an increased blood flow through the brain which would account for the fall in the arterio-venous oxygen differences. Inasmuch as our determinations as well as those of Himwich *et al.* (5), were made during actual coma, approximately 4 hours after insulin injection, and the observations of Abramson *et al.*, were made between 60 and 115 min. after injection, our data are not comparable. The latter authors state: "For the most, the various symptoms associated with hypoglycemia appeared only after an increase in peripheral blood flow was noted." Moreover, to explain a fall of the average arterio-venous difference from 7 to 2 vol. per cent, an increase of the rate of blood flow of 350 per cent would be required. It is doubtful whether so great a change could occur in the brain blood flow under these conditions. Direct measurements of cerebral blood flow during hypoglycemia reveal no such changes. In man a gradual slight decrease of brain blood flow during hypoglycemia has been reported by Loman and Myerson (21). Such a result is in agreement with the fact that there is a gradual fall in blood pressure as the hypoglycemia develops. Studies by Leibel and Hall (22) on rabbits during hypoglycemia reveal no significant changes in blood flow unless convulsions ensue. Because of these direct observations of a decreased or unchanged brain blood flow, and because of the fact that the patients become comatose, we believe that the smaller arterio-venous differences indicate a diminished brain metabolism.

In the present experiments evidence has been presented indicating that the brain tissue *in vivo* cannot oxidize lactic acid sufficiently to sustain cerebral function. In the hepatectomized animals the administration of lactate

did not serve to alleviate the hypoglycemic convulsions at once. These results are in agreement with those of Mann and Magath (4). Similarly, the comatose state associated with insulin hypoglycemia in man could not be relieved by the administration of lactate. The oxygen uptake of the brain in man, which is diminished in hypoglycemic coma, was only slightly increased despite the fact that large amounts of lactate were removed from the blood. It is well known that the liver may convert lactate to glycogen (6) which, after conversion to glucose, may serve to elevate the blood sugar. This mechanism for the glucose lactic acid cycle functions with high levels of blood lactate under postabsorptive conditions (24) but the rate of liberation of glucose by the liver is not sufficiently great to cope with the cerebral requirement for glucose. On the other hand, after hypoglycemia of 5 hours duration, Himwich *et al.* (23) found that the injection of 4 g. of glucose diminished the depth of coma and 8 g. of glucose caused the reappearance of both consciousness and the alpha waves of the electroencephalogram. In a separate group treated at Bellevue Hospital, 4 g. of glucose invariably roused the patient after the onset of coma, and restored the oxygen uptake from 2.9 to 5.5 vol. per cent approximating the precomatose levels (26). This is contrary to the evidence derived from the studies of oxidations of excised brain tissues. The possibility remains, however, that the oxidations *in vitro* occur at a diminished rate (25) and may be maintained by lactate oxidations, while the oxidation of lactate *in vivo* is too slow to support cerebral metabolism during hypoglycemia.

Summary and Conclusions

Twenty grams of r-sodium lactate were injected into patients during therapeutic hypoglycemic insulin coma. The injected lactate did not support brain oxidations sufficiently to rouse the patients, and the oxygen uptake of the brain showed only a moderate increase.

Seven dogs were hepatectomized under ether anesthesia, and were studied after they recovered from the anesthesia. Lactic acid, whether formed by the animals or administered intravenously, did not serve to prevent hypoglycemic convulsions or to alleviate them once they were initiated. This failure contrasts strikingly with the effects of glucose and indicates that the rate of oxidation of lactic acid in the brain is not sufficient to adequately support cerebral functions.

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CORTICAL EXTINCTION IN CONVULSIONS*

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INTRODUCTION

THE PURPOSE of the present communication is to demonstrate that (i) following an epileptiform seizure, whether induced by electrical stimulation or occurring "spontaneously," the electrogram of the human cerebral cortex discloses for a period a condition predominantly isoelectric; (ii) during this period of isoelectricity a stimulus of supraliminal value applied to the cortex is ineffective for the production of changes (a) in somatic motor activity and (b) in the cortical electric potentials; (iii) the slow restoration of cortical excitability (as measured by the motor response to stimulation) is signalled by a displacement of the isoelectric state; and (iv) an exalted state of cortical excitability probably then supervenes. These phenomena have not been recorded heretofore. That the qualitative and quantitative data derived from the present inquiry closely resemble or are identical with those of "extinction," as demonstrated in the less vigorous physiological activity of the subhuman nervous system, will become evident as the experimental findings are described.

The phenomenon known as *extinction* was discovered in 1934 by Dusser de Barenne and McCulloch (6). Its demonstration has proven of value to neurophysiology, contributing notably to progress in the study of the processes of neural decrement, inhibition, fatigue and oscillation just as the demonstration of facilitation by Bubnoff and Heidenhain (3) in 1881 contributed to an understanding of the processes of excitation. As defined, extinction is a diminution or absence of response observable (within an interval longer than that required for facilitation) upon repetition of stimulation of a "motor" focus of the central nervous system (9). Its influence may be observed as early as from several seconds to one-half a minute after the application of the stimulus responsible for its initiation and it may continue to exert its effect in some cases for as long as half an hour. Thus, a stimulus applied to a physiologically circumscribed region, *e.g.*, the hand moiety of the premotor cortex in Brodmann's area 6aα, so alters the functional condition of the central nervous system that when later a test stimulus is reapplied to that region, the measured response is either diminished or completely extinguished. A similar diminution or absence of response is observable when the test stimulus is applied to the immediately adjacent cortex and to cortical regions spatially more remote but functionally related to the hand region (*e.g.*, in the corresponding regions of Areas 4, 3, 1, 2 and 5). The diminution in response referred to may be manifest as a rise in threshold of excitability, an increase in the latent period between the application of

* Awarded the William Browning Prize of the Associated Physicians of Long Island, 1941.

stimulus and the first appearance of a measurable response, a decrease in amplitude or response, or any combination of these.* Extinction obtains as well with stimuli of subliminal value as with those of supraliminal value and in this respect it is comparable to the phenomena of summation and occlusion as they relate to facilitation. The more recent experiments of Dusser de Barenne, McCulloch and their associates (7, 8, 9) clearly demonstrate that the following factors attend extinction: (i) hypoactivity resulting in less summation; (ii) positive voltage drift associated with increase of threshold in neurones previously involved; and (iii) acid shift (decrease of pH), increasing the threshold of all neural structures in the region involved.

The observations presented here relating to seizure potentials of the exposed human brain were begun in September, 1938. They were originally undertaken with the intention of seeking a method which might supplement and render more precise the data obtainable from other sources bearing on the localization of epileptogenous foci. Prior to this time, it had been the frequent experience of the writer at operation that, following a convulsive seizure elicited by an electrical stimulus of relatively low strength, the cortex remains for a variable period of time quite unresponsive to the reapplication of a stimulus of the same or higher value. An attempt was therefore made in the present inquiry to obtain quantitative information relative to the two major types of change which are produced by antecedent stimulation, namely, alteration in threshold, as revealed by the determination of the minimal stimulation which produces an electrical response at the region under study; and alteration in activity as revealed by the electrical potential records of the cortex.

The number of studies in which the bioelectric potentials of the exposed human brain have been recorded for any purpose is unusually small (13, 23, 24, 26, 27). Foerster and Altenburger (13) were the first and seemingly the only investigators to record from the exposed human cortex electrical potential changes incidental to a seizure.

The literature discloses a few observations which bear more or less directly on the problem on hand. Thus, the experiments of Fischer (12), and of Goodwin, Kerr and Lawson (21) on the exposed animal brain and the observations of Berger (2), Gibbs, Davis and Lennox (16, 17, 20) and Davis and Sulzbach (5) on man with intact cranium demonstrate that following both drug-induced and spontaneous seizures the cerebral potentials are for a period "flat." This finding came to be interpreted as representing a decrease in bioelectric activity, running more or less parallel with the subsidence of muscular activity and prevailing for a period corresponding roughly to the duration of post-convulsive stupor. In addition to these findings, the observations of Fender (11) on dogs and of Clark and Ward (4) on cats coincide generally with the observations of the present writer to the effect

* It is of course essential that the reader bear in mind the physiological characteristics which differentiate *refractory period*, *suppression* and *inhibition* (in Sherrington's sense of the term) from *extinction*.

that cortical stimulation at intervals after an induced seizure yields "less complete seizures" even though a stronger stimulus than that which first proved effective was employed. Quantitative data were not recorded in these animal experiments.

MATERIALS AND METHODS

Ten human subjects constitute the basis of the present study. Seven were males and three females. The age of the youngest patient (J.C.) was $12\frac{1}{2}$ and of the eldest (F.O.) 32 years. All experiments were performed during the course of operative procedures for paroxysmal convulsive disorders at the Kings County Hospital.

On the basis of preliminary studies consisting of the anamnesis, the characteristic sequence of events of the seizure pattern, the neurological findings, roentgenography, pneumoencephalography and electroencephalography, the site of the epileptogenous focus was postulated in each case. Sedatives were avoided during the two days before operation. The scalp was infiltrated with procain hydrochloride, an osteoplastic bone flap was turned down and the dura reflected. A careful sketch of the disposition of the sulci, gyri, cortical vessels and sites of pathological alteration (if any) was made and some seven to ten "zones" (depending upon the magnitude of the area of the exposed cortex) were arbitrarily defined on the cortical surface as points of reference. These zones were indicated on the sketch.

A control electrographic record was now made for each separate zone, the electrical potentials being picked up by means of electrodes and a holder especially designed for this purpose. Under certain conditions, the familiar co-axial type of electrode of Adrian and Bronk (1) was used. The brain potentials were led through a single-channel electroencephalograph and, after amplification, were recorded simultaneously by two means, *viz.*, a cathode ray oscillograph, the standing waves of the sweep circuit of which could be observed by the surgeon directly and the oscillations of which could be recorded cinematographically; and a push-pull type of ink-writing oscillograph, the oscillations of which could be recorded directly on a moving paper tape. The basic design of this instrument has been described by Garceau and Davis (14, 15).

After obtaining control records of the various zones in the manner above described, an attempt was made to identify more precisely the epileptogenous focus ("firing point") presumed to lie within the operative field. For this purpose stimulation of the cortical surface was carried out beginning with values well below threshold and working up by successive increments until the limen for eliciting a motor response from the excitable motor cortex of the face and/or hand area was ascertained. The exposed cortex was then systematically explored with this strength of stimulus, the intention being to discover a region from which a seizure could be produced that simulated in its subjective and objective features the so-called spontaneous seizures of the patient. If this stimulus value failed to produce a response it was gradually increased until the desired result was obtained. The instrument employed for this purpose was a 60 c./sec. bipolar stimulator. In the first 4 experiments an A.C. sinusoidal wave form having a period of 16.6 msec. was used. In the other 6 experiments an interrupted D.C. parabolic type of wave having a period of 8.3 msec. was employed through the intermediation of a copper rectifier. The bipolar stimulating tips consisted of silver balls of 1.5 mm. diameter separated 2–3 mm. from each other. The stimulating tips were applied for approximately 3 sec. An interval of 10–14 sec. was allowed to elapse between successive applications of the stimulating electrodes to the cortical surface, the purpose of this delay being to permit the effects of induction and facilitation to pass off.

A trained observer was posted under the sterile drapes for the purpose of reporting to the surgeon the patient's responses to stimulation. All observations were recorded stenographically and were correlated with the electrographic tracings, particular attention being given to temporal factors. After each application of a stimulus, the pick-up electrodes were applied to the corresponding cortical zone. Whenever a seizure was initiated, the electrographic record was continued until an effectually normal tracing of cortical potentials reappeared. Meanwhile, the effect of reapplying stimuli of varying supraliminal as well as liminal strengths was noted at regular intervals.

Results

The essential conformity of the descriptive data derived from the 10 experiments permits a treatment of the series as a whole. In the interest of space conservation, therefore, the findings are summarized in Table 1. Only those protocols (or portions thereof) will be presented which appear to be

Table 1. Summary of the data derived from the 10 experiments of the present series bearing on the phenomena of cortical extinction and isoelectric state. (Period of extinction includes both absolute and relative phases; Ineffective refers to failure to produce a major convulsion; Undet. = undetermined.)

Exp't No.	Patient	Value of stimulus evoking convulsion	Duration of period of extinction	Highest value of stimulus ineffective during extinction	Exalted state following extinction
I	J. C.	0.65 mA. 4.5 V.	19 min.	2.25 mA. 9.0 V.	Yes
II	W. G.	1.5 mA. 6.5 V.	11 min.	2.25 mA. 9.0 V.	Undet.
III	H. H.	1.0 mA. 5.5 V.	21 min.	2.10 mA. 8.5 V.	Undet.
IV	T. C.	0.45 mA. 4.0 V.	25 min.	2.5 mA. 10.0 V.	Yes
V	M. W.	0.20 mA. 2.5 V.	14 min.	2.1 mA. 8.5 V.	No
VI	P. B.	0.10 mA. 1.0 V.	21 min.	2.35 mA. 9.5 V.	Yes
VII	M. R.	1.30 mA. 6.0 V.	9 min.	2.35 mA 9.5 V.	Undet.
VIII	A. P.	0.65 mA. 4.5 V.	17 min.	2.25 mA. 9.0 V.	No
IX	F. O.	2.25 mA. 9.0 V.	19 min.	3.25 mA. 13.0 V.	Yes
X	J. S.	1.7 mA. 7.0 V.	22 min.	2.35 mA. 9.5 V.	Undet.

of direct value in supplementing the table and in illustrating the major points under consideration in this communication.

Experiment I. Patient J. C., a schoolboy aged 12½ years, was admitted for the second time to the Kings County Hospital on September 8, 1938. The neurological findings, the sequence of events of a typical seizure and the electroencephalographic tracings appeared to justify the supposition that an epileptogenous firing point might be discoverable high on the convexity of the right hemisphere in either Area 6 or Areas 3, 1, 2, 5 or 7. Accordingly on September 26, 1938 a bone flap was reflected. The exposed cortex, substantially normal to gross inspection, presented an appearance as illustrated in Fig. 1. A small focal area of

cortical atrophy with a correspondingly deep subarachnoid lake was noted near the junction of the precentral sulcus and the posterior limit of the middle frontal convolution. Cortical electrograms were recorded from each of the 9 zones (Fig. 1) after which stimulation was begun. No responses were obtained until a stimulus of 0.12 mA. and 1.5 V. was

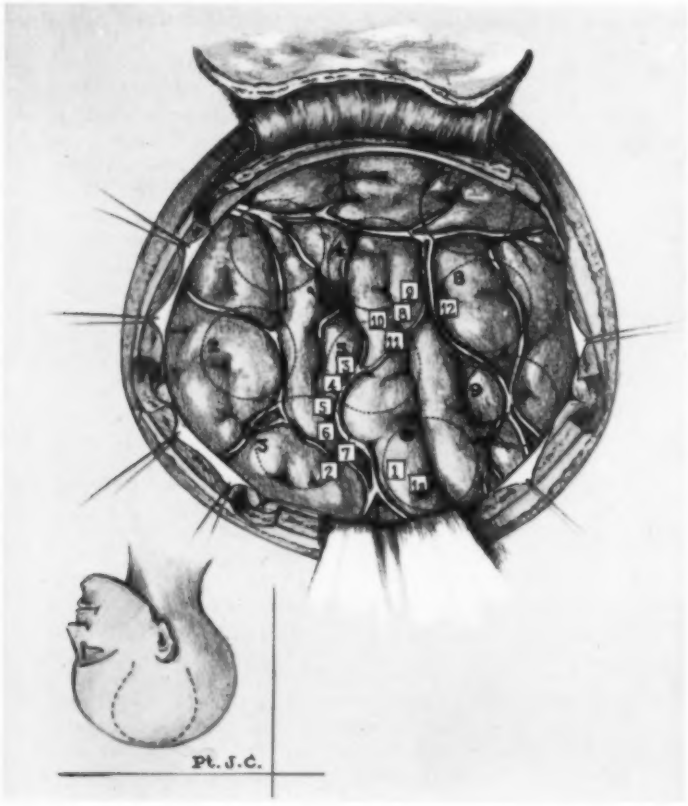

FIG. 1. The operative field of patient J. C. The numbers within the dotted circles indicate the 9 arbitrary zones from which control electrograms were recorded. The arrow points to a small region of cortical atrophy. The numbers within the squares indicate the points from which responses described in the protocol were obtained.

employed. This stimulus was everywhere ineffective except at the point marked 1. From here, movements of the left upper extremity closely simulating those characteristic of the earliest motor events of the patient's spontaneous seizures and lasting 12 sec. were elicited. This stimulus and stimuli at graded increments ranging up to 1.5 mA. and 6.5 V. were wholly ineffective during the next 17 min. when applied at intervals of 14 sec. to all parts of the exposed cortex. At the end of this time, the originally effective stimulus again proved capable at point 1 of exciting a response, this time of 5 sec. duration, in the left upper extremity. The pattern of this response was as previously observed. The stimulus was now set at 0.30 mA. and 3.5 V. The cortex in and adjacent to point 1 was purposely avoided until the other parts of the cortex should be explored. At the point marked 2 the patient reported experiencing "a feeling as if I'm going to go." There was a slight and brief movement of flexion of all fingers of the left hand but the episode was arrested here. This same value of stimulus proved effective for the elicitation of slow tonic flexion movements of the

individual digits when it was applied to the points marked 3, 4, 5, 6 and 7 in Fig. 1. Restimulation of each of these points at an interval of 1.5 min. following the first reaction elicited a response of slightly less magnitude. The same strength of stimulus was now applied to the point marked 8 where it evoked a quivering of the lower lip of 3–4 sec. duration. As soon as the quivering ceased, the point was restimulated and a like response was again observed. This time, however, it lasted for 7 sec. (facilitation). Reapplication of the stimulus to region 8 and to the surrounding regions (9, 10, 11, 12) was without effect 5 min. later.

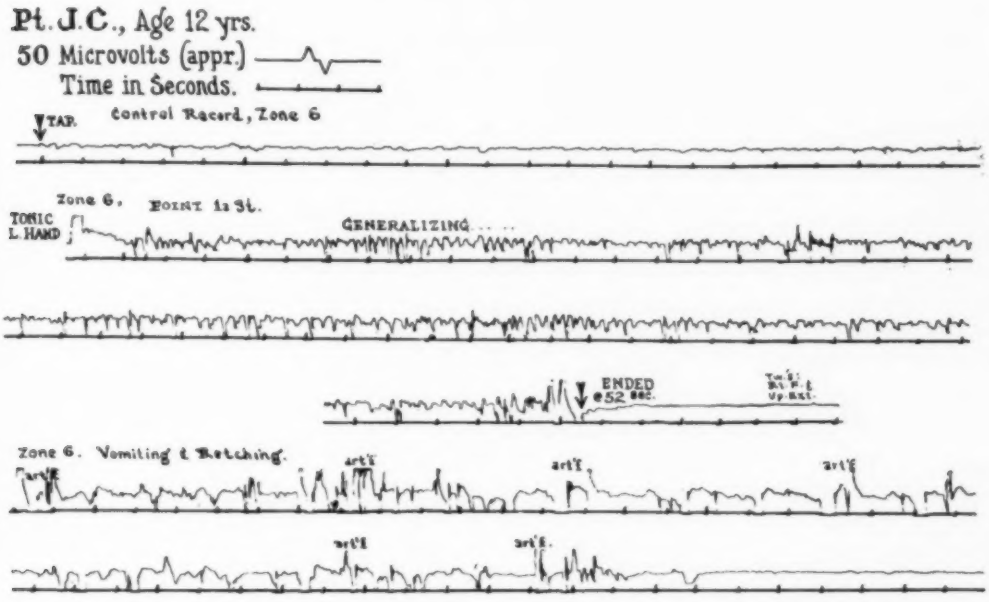

FIG. 2. Cortical electrogram from zone 6. Stimulation at point 1a with 0.65 mA. and 4.5 V. elicited a convulsion starting with a tonic contraction of the left hand and marching into a generalized seizure. The duration of the electrically recorded seizure was 52 sec. During the ensuing isoelectric period twitches of the right face and upper extremity were still observable (tw's: rt. f. & up. ext.). The tracings during retching and vomiting and the succeeding isoelectric state are shown (art'f. = artifacts due to gross head movements).

The stimulus values were now advanced to 0.65 mA. and 4.5 V. and the cortex was again explored, avoiding at the beginning the regions from which the above described responses were obtained. When the stimulus was applied to point 1a, a major convulsion supervened the pattern of which closely simulated the spontaneous attacks of the patient. An electrographic tracing of zone 6 was made beginning at the moment when the tonic extension of the left hand was reported. The electrically-recorded attack lasted for 52 sec. In spite of the fact that following this attack the tracings from zone 6 appeared isoelectric, a few irregular twitches of the right face and right upper extremity were observable during the next 8–9 sec. The isoelectric state persisted for some 12–13 sec. and then the patient began to retch and vomit. The record obtained at this time was irregularly interlarded by readily recognizable artifacts, obviously due to coarse movements of the head. As soon as the retching subsided, however, the condition of essential isoelectricity again prevailed (Fig. 2).

During the immediately succeeding 1.5 min. that strength of stimulus which evoked the described major convulsion proved wholly ineffective when applied to all points previously responsive, including 1 and 1a. At the end of 2.5 min. a stimulus of 2.25 mA. and 9.0 V. applied to point 1 elicited a very weak response consisting of abduction of the left arm and flexion of the left forearm and wrist (Fig. 3). No clonic features were noted in this action.

The patient was now restless and occasionally moaned and mumbled. The stimulus was reduced to its previously liminal value of 0.65 mA. and 4.5 V. It now proved ineffective when applied at intervals of 1 min. to the cortex in and around point 1 until the seventh minute following the termination of the convulsion. The cortical tracings at this time were still relatively isoelectric, but a response was nevertheless obtained, consisting of a progressive tonic spread of the fingers of the left hand, an extension of the hand and an abduction movement of the arm. This tonic action lasted for 1–2 sec. and was followed by a few clonic jerks, the entire episode lasting approximately 5 sec. The electrogram disclosed a corresponding interruption of the isoelectric tracing (Fig. 3). Evidence of increasing cortical

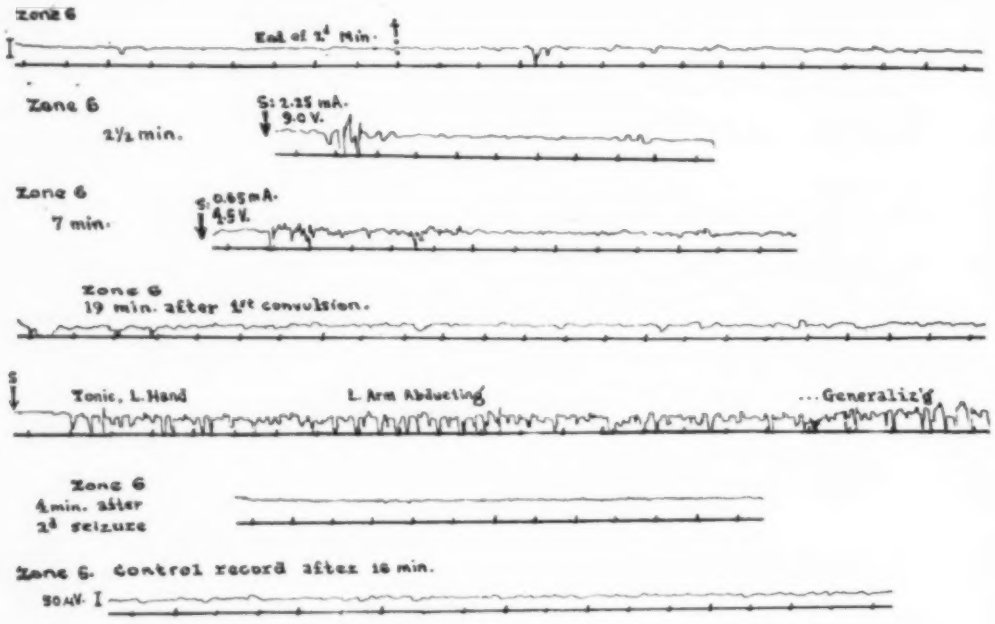

FIG. 3. Cortical electrograms from zone 6. The prevailing isoelectric state following the convulsion of Fig. 2 beings to break (right half of top line). Following the seizure by 2.5 min. application of a supraliminal stimulus of 2.25 mA. and 9.0 V. to point 1 elicited a brief and weak response of the left upper extremity. At the end of 7 min. the liminal value of stimulus, 0.65 mA. and 4.5 V., excited from point 1 an "aborted" attack lasting 5 sec. At the 19th minute, autonomous potentials closely resembling those of the control period entirely replaced the isoelectric tracing. At this time, the liminal value of stimulus elicited a major spell of greater severity than the first, lasting 88 sec. Only the earliest part of the tracing is shown here. A sample of the isoelectric tracing taken 4 min. after the second convulsion follows. The lowermost strip demonstrates returning potentials 16 min. after the attack.

excitability was obtained from this time on and by the fifteenth minute more nearly autonomous tracings began to appear. When, at the nineteenth minute, the latter appeared to be well established, the originally liminal stimulus (*i.e.*, 0.65 mA. and 4.5 V.) was applied to point 1 and this time a second major convulsion was evoked which, in its pattern, closely simulated that first elicited. From the clinical standpoint this attack was more severe than the first, as judged by its greater duration (88 sec.) and by the vigor and amplitude of the convulsive movements. After the cessation of this seizure, an isoelectric state again prevailed not only in zone 6 but in the other zones of the exposed vortex (Fig. 3). Sixteen minutes later, the electrogram revealed a return of more nearly normal autonomous potentials.

Experiment IV. Patient T. C., a plumber aged 28 years, was admitted for the second time to the Kings County Hospital on November 9, 1938 with a history of convulsions refractory to medical treatment for 11 months. The attacks invariably began with persistent twitching of the left eyelids and were either aborted here or progressed thence in a physiologically orderly march into a generalized convulsion. Electroencephalography disclosed a dysrhythmia of the right frontal and temporal regions the pattern of which did

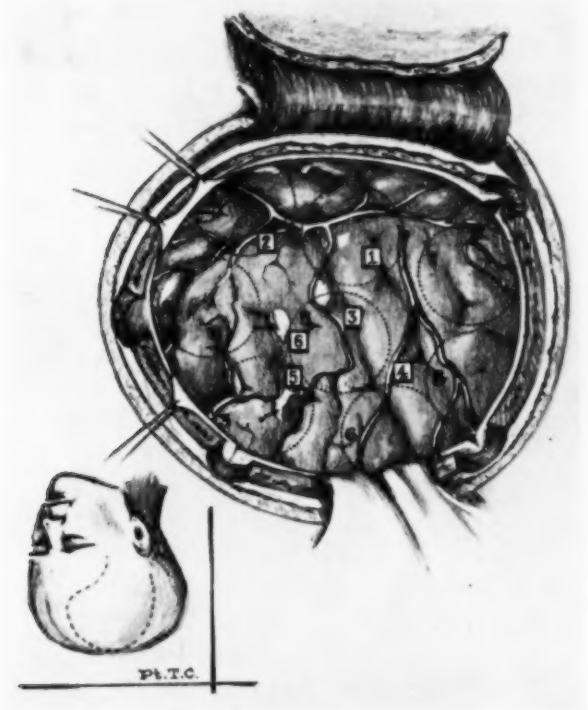

FIG. 4. The operative field of patient T. C. The numbers within the dotted circles indicate the 8 arbitrary zones from which control electrograms were recorded. The 2 arrows point to small opaque corticoarachnoid cicatrices. The numbers within the squares indicate the points from which responses described in the protocol were obtained.

not conform closely to any well defined category (17, 18, 19, 20, 22, 25). The sum total of clinical evidence was suggestive of a firing point in Area 8b of the right frontal lobe. On November 22, 1938 a right-sided bone flap was reflected. The cortex appeared essentially normal except for a dense, opaque sclerotic patch located at the junction of the precentral and inferior frontal gyri. This seemed to draw a number of the local vessels into a pit. A similar patch was noted 1.5–2 cm. superior to the first (Fig. 4). Control electrograms were made of the eight zones indicated on the sketch and stimulation was begun with 0.20 mA. and 2.5 V. No responses were elicited until these values were increased to 1.0 mA. and 5.5. V. At point 1 this strength of stimulus elicited a twitching of the upper and lower lips on the left side which persisted for 55 sec. The left eyeball went through irregular rhythmic jerks at this time, seemingly independent of its fellow. Five minutes later the same stimulus, when applied to point 2, elicited twitching movements of the left forehead, cheek and eyelids and a clonus of the platysma myoides. This response lasted but 25 sec. At the onset

the patient reported a sensation "as if" he were turning. The stimulus was now reduced to 0.45 mA. and 4.0 V. At point 3, fluttering movements of the left eyelids were produced, succeeded after 9 sec. by a few clonic jerks of the left forearm of 11 sec. duration (Fig. 5). Stimulation of the same point at the end of 2 min. resulted in only a few fluttering motions of the left eyelids, the episode lasting but 8 sec. Ten minutes later the same stimulus when applied to point 4 elicited clonic twitches which appeared almost simultaneously along the left upper extremity, from the fingers to the shoulder. After another 10 min., stimulation at point 5 evoked a seizure which closely simulated the patient's usual attacks. The episode lasted 2.5 min. The electrogram disclosed oscillations of high frequency and large amplitude, gradually becoming isoelectric (Fig. 6). Systematic stimulation of the exposed brain during the ensuing 22 min. was entirely ineffective with the same stimulus and with stimuli ranging up to 2.5 mA. and 10 V. The electrogram now began to show an irregular return of

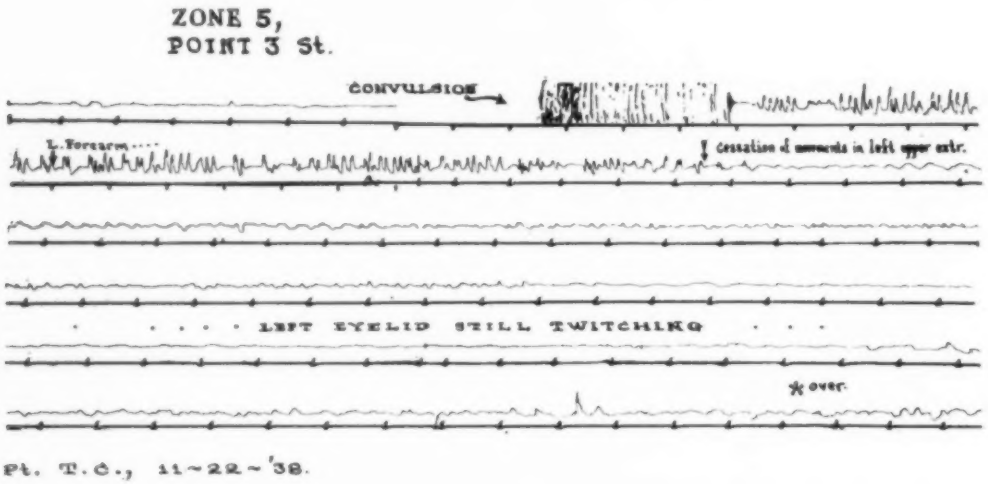
ZONE 5,
POINT 3 St.
CONVULSION
L. Forearm . . . .
* cessation of movements in left upper extr.
. . . . . LEFT EYELID STILL TWITCHING . . .
* over
Pt. T.C., 11-22-'38.

FIG. 5. Cortical electrogram from zone 5. Stimulation at point 3 with 0.45 mA. and 4.0 V. evoked fluttering movements of left eyelids followed after 9 sec. by irregular clonic jerks of the left forearm (L. Forearm . . .). Note eyelids still twitching during a prevailing isoelectric state in this zone. Asterisk denotes cessation of all observable twitching.

autonomous oscillations. Therefore the stimulus values were reduced again to the previously determined liminal values of 0.45 mA. and 4.0 V. At the twenty-fifth minute, this stimulus, elsewhere ineffective, was in the process of approaching point 5. When it was applied to point 6 a severe convulsion, far more violent in the vigor and amplitude of its clinical features than the previous seizure, was evoked. Again, a period characterized by an isoelectric state supervened (Fig. 7). This state was altered at times by slow voltage drifts and was replaced after 19 min. by more nearly normal autonomous waves. At the twentieth minute following this attack, while observations of the post-convulsive period were still being made, a "spontaneous" seizure appeared, lasting 65 sec. This was of the same pattern as noted in the two previous attacks, but clinically it appeared to be the most severe of all. The cortical electrogram corresponding to the succeeding post-convulsive period disclosed an isoelectric state, demonstrating that this condition of the cortex follows "spontaneous" as well as induced seizures (Fig. 7).

Experiment V. Patient M. W., a laborer aged 31 years, was admitted to the Kings County Hospital on March 29, 1939 complaining of convulsive seizures of 21 years' duration. The onset of the attacks was characterized by electric-like shocks in the right hand and forearm, numbness and tingling of all fingers of this limb, and severe clonus of the limb. The arm then twisted behind the back, the head rotated to the right and the right eyelids twitched. The attack was either aborted here, in which case consciousness was not lost, or it became rapidly generalized, in which case unconsciousness supervened. Further details

FIG. 6. Cortical electrogram from zone 3, 22 min. after response recorded in Fig. 5. Stimulation at point 5 with 0.45 mA. and 4.0 V. elicited a seizure simulating the patient's usual attack. Liminal and supraliminal stimuli were ineffective during the succeeding 22 min.

of the work-up of this case will be foregone inasmuch as they are not relevant to the point it is desired to illustrate here. At operation on May 9, 1939 a left osteoplastic bone flap was reflected. The opercular region presented a dense broad cicatricial sheet, the sequel of an old cerebral contusion. The anterior edge of the scar proved to be the region from which,

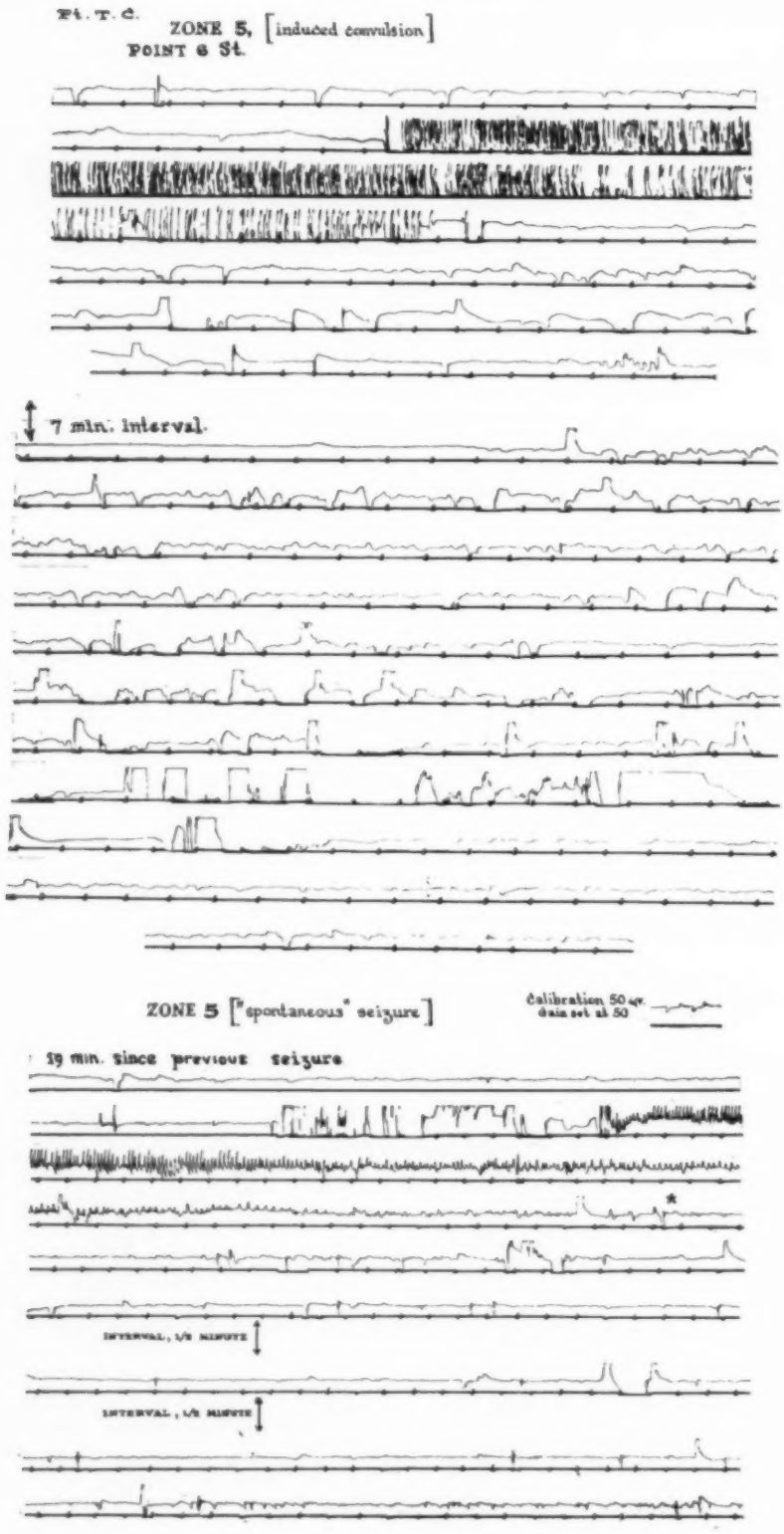

FIG. 7. *See next page for legend.*

FIG. 7. Cortical electrogram from zone 5, 25 min. after the attack recorded in Fig. 6. Stimulation at point 6 with 0.45 mA. and 4.0 V. elicited a major seizure of greater severity than that previously evoked. Twenty minutes later a spontaneous seizure supervened, the most severe of all. Note prevailing isoelectric state following the latter attack.

with a stimulus of low strength (0.20 mA. and 2.5 V.) a convulsion closely simulating the patient's spontaneous attacks was elicitable. As indicated on the record, during the isoelectric period which followed this convulsion twitchings of the left side of the face were still observable (Fig. 8). After the complete subsidence of the convulsion and throughout the succeeding 14 min. stimuli of values ranging as high as 2.1 mA. and 8.5 V. proved inade-

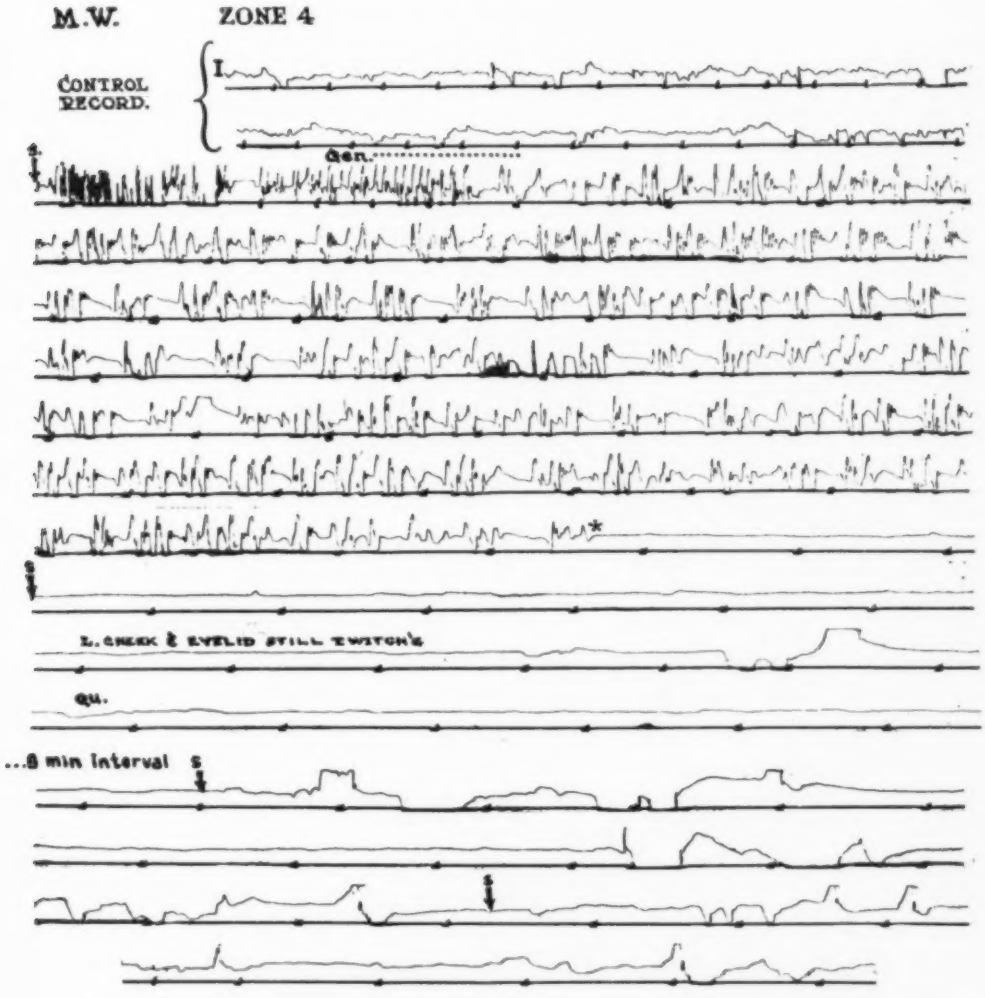

FIG. 8. Cortical electrogram from patient M. W., approximately in Brodmann's area 44. Convulsion elicited by stimulation of point on anterior edge of cicatrix, using 0.20 mA. and 2.5 V. Note that during the isoelectric state, twitches of left cheek and eyelids were still observable. I = 50μ V. deflection; S = application of stimulus; Gen. . . . = convulsion becoming generalized; asterisk denotes end of generalized convulsion; Qu. = entirely quiet.

quate to excite an evident motor or sensory response. The point to which particular attention is drawn by this experiment (as also by experiments I and II) is that motor responses were still observable at a time when the electrogram of the zone about the stimulated point appeared isoelectric. It seems reasonable to suppose that an electrogram taken from the homologous zone in the opposite hemisphere at this time would have disclosed a few waves of large voltage and rapid rate. Under the conditions of experimentation, however, the recording of such potential changes was not feasible.

Discussion

The protocols are consistent from experiment to experiment and they appear to demonstrate the following points:

1. The strength of stimulus required to evoke a major convulsive seizure by stimulation of a "firing point" varies from case to case within rather wide limits (from less than 0.10 mA. and 1.0 V. to 1.2 mA. and 9.0 V.). This variation is such that in one patient a convulsion may be discharged by a stimulus the value of which is insufficient to excite even a simple response when applied to the motor cortex of another patient.

2. The strength of stimulus required to evoke a major convulsive seizure by stimulation of a "firing point" can be shown to vary from attack to attack in any given patient during the course of an operation. This variation is at least in large part if not entirely ascribable to the antecedent activity of the cortex. (a) The application of the stimulating electrodes carrying liminal and supraliminal values proved ineffective to excite a convulsion for an average of 17.8 min. after the offset of a previously elicited attack. (b) During the advancing course of this period, motor responses of a delimited order, usually characterized by the earliest events of the spontaneous seizure pattern and resembling the aurae and aborted seizures of the patient, can be elicited provided a stimulus of sufficiently high supraliminal value is employed. As the period progresses, a supraliminal stimulus of a given value elicits more and more vigorous responses or, expressed in another way, the supraliminal value required to evoke a response of given magnitude is in inverse proportion to the time. (c) Following the expiration of this period, stimuli of original liminal value are seemingly capable of eliciting a convulsive seizure of more vigorous character than that first evoked. This was apparent in four of the six experiments in which its demonstration was specifically sought. The two negative results indicate the necessity for reserving final conviction in this regard until further experimental data are at hand. It is conceivable, however, that in these two experiments the stimulus which proved capable of eliciting a second major convulsion was applied to the neighborhood of the firing point at a time somewhat too late to find the cortex still in an exalted state of excitability. In the other four cases attempts to demonstrate the phenomenon had to be abandoned for one reason or another dictated by the circumstances at operation.

3. The period during which the convulsive responses are either wholly incapable of elicitation or appreciably dampened is characterized by a relatively isoelectric state as revealed by the cortical electrograms. As the period progresses, the electrographic tracings indicate an irregular resumption of

cortical potentials which come more and more to resemble those observed during the control period. It appears that the electrical excitability of the cortex is recovered *pari-passu* with the subsidence of the isoelectric period until it at last becomes such that a second convulsion is elicitable. In this series the periods of absent or diminished response ranged between 9 and 25 min. and they were observable following both spontaneous and induced seizures. They could not be said to correspond precisely to the period of depressed psychological responsiveness (clinically, "unconsciousness," "post-convulsive stupor," etc.) inasmuch as fairly complex verbal responses were executed in some instances at the time when a relatively isoelectric state prevailed.

The evidence thus far arrived at appears to indicate that the phenomena underlying and following upon the discharge of a convulsion are qualitatively identical with those underlying other more typical physiological excitations. It therefore seems unnecessary to invoke any new principle in attempts to account for the spread of excitation in seizures, such as an electrical wave front advancing across the cortical surface without reference to neuronal interconnections, a vasospastic process, a hypothetical chemo-excitant, etc. On this point the writer concurs with Erickson (10) who adduced strong experimental evidence to the effect that the spread of cortical excitation in convulsions is mediated strictly by neurones and their processes.

Whether a positive voltage drift, a decrease in pH and an accumulation of lactic acid in the cortex are regular concomitants of the post-convulsive isoelectric state in the human will have to await demonstration until methods recently evolved in the animal laboratory can be adapted to the operating room. Nevertheless, the phenomena associated with the period of increased threshold observed in the present experiments resemble so closely those of extinction that eventual proof of their identity appears to be reasonably certain.

4. The exalted state of cortical excitability which seemingly follows the period of extinction in certain experiments is of additional interest. It suggests a partial account for the clinically described *status epilepticus*, *viz.*, after a convulsion is spent, there supervenes a period during which the cortex is unresponsive to the substrate of physiological stimuli (*e.g.*, hypoglycemia, alkalosis, anoxia, positive water balance, endocrine factors, etc., etc.) which tend to set off a new spontaneous attack; as the state of cortical excitability passes from one of absolute extinction to one of relative or partial extinction a second attack (of weaker character) may be discharged, provided only that the stimuli are still "set" at a sufficiently high level; if they are not so set they remain incapable of discharging a new seizure until the exalted state of cortical excitability following the period of extinction is reached. The process is then repeated. According to such a concept, *status epilepticus* can be terminated only by one of two mechanisms: (i) by the development of a more enduring depression of the excitability of the cortex, (a) "spontaneously" through repetition of the above cyclical events or (b) by the use

of drugs and other anti-convulsant agents; and (ii) by the subsidence of the tide of physiological stimuli which initiated the status epilepticus, (a) "spontaneously" or (b) by properly directed therapy. If these hypothetical considerations should prove defensible, the significance of the period of extinction to status epilepticus could be held to account similarly for the more common parallel phenomena of a single convulsive episode.

Summary

1. A major convulsion is followed by a period of cortical extinction which is demonstrable (a) by the unresponsiveness of the cortex to liminal and supraliminal stimuli and (b) by the essentially isoelectric character of the cortical electrogram. The evidence from this source tends to support the contention that the phenomena which underlie convulsive seizures are qualitatively identical with those of other more typically physiological processes of excitation.

2. The restoration of cortical excitability is indicated by a slow and irregular subsidence of the isoelectric character of the electrographic tracings and by the return of cortical potentials which resemble those of the control period. Supraliminal stimuli applied to the firing point may now elicit responses resembling an aborted spontaneous seizure.

3. For a brief period following the offset of the period of extinction, the cortex in the neighborhood of the epileptogenous focus may be in an exalted state of excitability and at this time a liminal stimulus may evoke a seizure of greater violence than that which characterized the first seizure.

4. The evidence suggests a physiological account for certain phenomena observable in the clinical syndrome known as status epilepticus as well as in the more sporadic single convulsions.

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INFLUENCE OF FREQUENCY OF STIMULUS UPON RESPONSE TO HYPOTHALAMIC STIMULATION*

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FREQUENCY OF stimulation may determine the type and magnitude of the vasomotor response to electrical stimulation of an afferent nerve (1, 2). When intensity and wave form are constant, alterations in the frequency of stimulation of an afferent nerve may reverse the direction of the blood pressure response in decerebrate or spinal cats (1). The integration of the vascular responses is therefore not dependent upon the cerebral cortex, the diencephalon, or the brain stem; and it follows that the activity of the spinal neurons may be modified by the rate at which other neurons discharge onto them.

Karplus and Kreidl (3) have demonstrated that electrical stimulation of the hypothalamus could influence spinal motoneurons, causing alterations in the activity of the autonomic nervous system. In the following experiment the hypothalamus has been electrically stimulated in 13 cats. When all other characteristics of the stimulus were constant, a change in frequency often, but not invariably, affected the respiratory, vasomotor, or pupillary response.

METHOD

The hypothalamus was stimulated with bipolar electrodes inserted into the brain and manipulated with a Horsley-Clarke apparatus. The electrodes were made by cementing together two pieces of enameled nichrome wire, after the technique of Ingram *et al.* (4). The stimulator used delivered condenser discharges of a constant voltage and wave form over a frequency range of 1 to 1600 per sec. (Fig. 1). During each experiment a calibration for frequency and voltage was made from photographs of an oscillographic recording and a D.C. amplifier was used to establish that the stimulus was free of any D.C. component. The voltage (3 to 6 V.) was frequently checked during each experiment by inspection of the oscillograph recording the output of the stimulator.

The spread of current at different frequencies was tested in two cats by carefully lowering the electrodes into the facial colliculus. The position was found which permitted stimulation of the abducens nucleus without stimulating the fibers in the overlying genu of the facial nerve. At a given frequency the voltage was gradually increased until the ipsilateral eye began to close. The voltage was reduced to its original value, the frequency changed and the process repeated. The voltage had to be increased as much at a frequency of 1100 per sec. as it did at 20 per sec. This was considered adequate evidence that the spread was not increased as the frequency was increased.

Blood pressure was recorded from the femoral artery with a mercury manometer using 1 per cent sodium citrate as an anticoagulant; respiration with a pleural cannula and a tambour; and time, in 3-second intervals, was recorded with a signal magnet so adjusted that the time line represents zero blood pressure. The rectal temperature was kept between 37 and 39°C. with an electric heating pad. Ether, nembutal, or chloralose were the anesthetics used.

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The hypothalamus was explored from the preoptic area rostrally, to the mesencephalon caudally, and as far as the internal capsule laterally. The more rostral and lateral punctures were usually made first in order to avoid damage to descending connections. The electrode was lowered 1 or 2 mm. at a time and a stimulus of 10 sec. duration applied at each stop. When a responsive area was encountered, the electrode carrier was firmly locked in position and a number of records taken with different frequencies of stimulation. During no recording was the electrode position or the voltage of the stimulus changed. When significant responses were obtained, the electrode in some cases was lowered no further, so that the bottom of the puncture could be identified as the area of excitation.

At the termination of the experiment, the cat was perfused with saline, then with 10 per cent formalin. The brain was removed from the skull and the diencephalon prepared for histological study which permitted identification of the hypothalamic areas stimulated.

Results

The results obtained are shown in the following recordings. They may be conveniently grouped into 2 categories: (i) those in which frequency changes reversed either the respiratory or blood pressure responses; and (ii) those in which frequency changes caused a change only in the magnitude of response. In all the figures the number below the signal line indicates the frequency of stimuli in impulses per second.

The first group is illustrated by Fig. 2, 3 and 4. These records show that a change in the frequency of stimulation may produce a reverse in response, while repetition of the same frequency produces responses similar in type and magnitude. Figures 3 and 4 show also that when a response to a given frequency consists of two components (a rise in blood pressure during stimulation, followed by a rebound fall), a different frequency may elicit only one of these components.

The second group is illustrated by Fig. 5, 6 and 7. Here no reversals were obtained, but enormous variations in the magnitude of the response followed stimulation of the same point with different frequencies. In some cases, frequencies above or below an optimal range caused diminished responses. Stimulation at a given frequency always elicited the same response. When stimuli of different frequencies were applied in a series, neither the order of the series, the length of the interval between stimuli, nor the time allowed between one series and the next affected the characteristic response to a given frequency.

In the absence of somatic movements, the size and type of the blood pressure response could be profoundly influenced by changes in the frequency of the stimulus. In two cats, after the skeletal muscles were inactivated by curare and artificial respiration was administered, changes in visceral responses attended alterations in the frequency of excitation. In the noncurarized cat, excessive respiratory movements were often associated with a change in blood pressure, but these movements were not the sole cause of the vascular responses. They persisted in the curarized animal.

Both dilatation and constriction of the pupils during hypothalamic stimulation, and in some experiments both responses, were obtained when the electrode was kept in the same position and frequency of stimulation varied. Low frequencies (1–50 per sec.) were more frequently associated

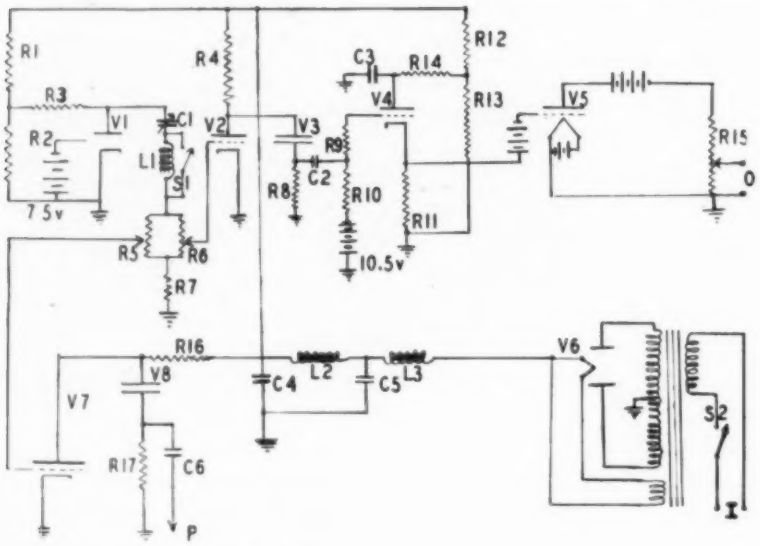

FIG. 1. Diagram of circuit of stimulator.

V1	885.	C1	Decade Condenser, .001 mf. to 1.11 mf. in .001 mf. steps.
V2	76.		
V3, V8	2 Watt neon bulb with resistor removed from base.	C2	.05 mf.
		C3	.003 mf.
V4	885.	C4	8 mf. electrolytic
V5	2 type 45 in parallel.	C5	8 mf. electrolytic
V6	80.		
V7	76.		
R1	100,000 Ω	L1	15 henry choke
R2	175,000 Ω	L2	20 henry choke
R3	100,000 Ω	L3	20 henry choke
R4	100,000 Ω		
R5	5,000 Ω pot.		
R6	5,000 Ω pot.		
R7	500 Ω	I	110 V., 60 cycles
R8	20,000 Ω	O	Output
R9	20,000 Ω	P	To Sweep-Oscillator Grid
R10	5,000 Ω		
R11	5,000 Ω		
R12	100,000 Ω		
R13	175,000 Ω		
R14	100,000 Ω		
R15	10,000 Ω pot.		

In this experiment, the circuit was operated with S1 closed, short circuiting L1. V1 operates as a relaxation oscillator; its frequency is determined by the value of C1. On each discharge of V1, a negative pulse appears on the grid of V2, driving the plate of V2 in a positive direction, and causing ionization of V3. At the moment of ionization of V3, a sharp positive pulse appears on the grid of V4. C3 has been previously charged to about 110 volts, but ionization of V4 has been prevented by the high negative bias on its grid. The sharp positive pulse on the grid of V4 allows C3 to discharge through it, but before discharge is complete, the high negative bias has been restored, and C3 recharges, awaiting another positive pulse on the grid of V4. V5 is biased to cut off so that no plate current flows except during the discharge of C3, when a positive pulse appears across R11. The shape and size of the output pulse depend only upon the conditions existing on V4 when a pulse is tripped. Adjustment of C1 varies the frequency of the oscillator V1, and thus varies the repetition

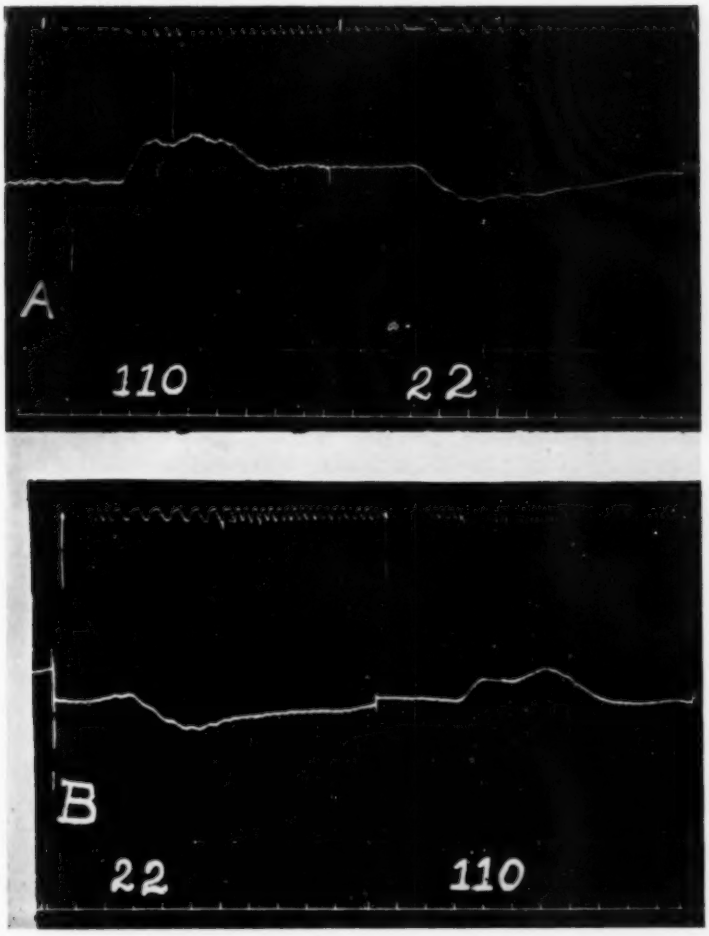

FIG. 2. Four records showing stimulation of the same point in the hypothalamus. a) Higher frequency, applied first, caused a rise in blood pressure and acceleration of respiration; lower frequency caused a fall in blood pressure and slowing of respiration. b) Sequence of stimuli reversed, and responses reversed. The electrodes were in the central grey at the rostral end of the midbrain.

rate of the tripping pulses. The output pulses are not strictly independent of frequency, since the higher the frequency, the earlier in the charging cycle of C3 tripping occurs. However, the charge on C3 is an exponential function of time and, over the range of frequencies used, tripping occurs near the asymptote, so that the variation of pulse size with frequency is very small. The difference in pulse size from one end of the range to the other could not be detected on oscillographic analysis.

The inductance L1 and vacuum tubes V2, V3, V7, and V8 are not essential to the operation of the circuit as used in this experiment; they are used in other applications to secure desired time relations between the stimulus and the tripping of a "single-sweep" circuit so that action potentials resulting from the stimuli may be observed as a standing wave. L1 broadens the discharge pulse of V1 so that it approximates the positive half of a sine wave. The points on this wave at which V3 and V8 ionize may be varied by adjusting R5 and R6, and a slight time lag between the start of the sweep and the application of the stimulus may thus be obtained. Where this feature is not required, the circuit of V1 could be arranged to deliver a positive pulse on discharge, and the pulse could be applied to the grid of V4 through a suitable resistance-capacity network without intermediate vacuum tubes.

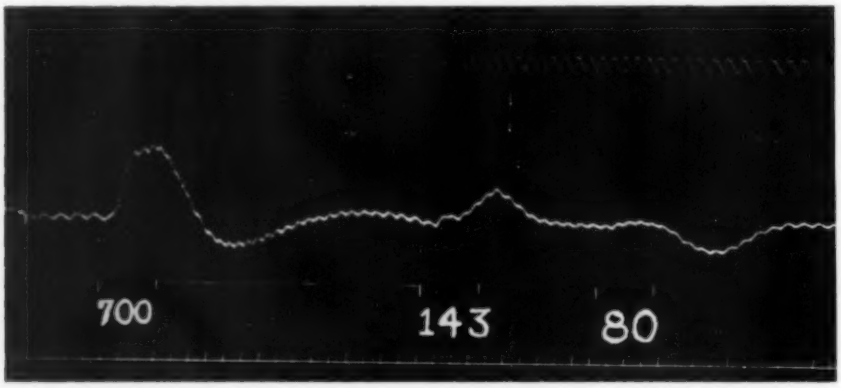

FIG. 3. First stimulus (highest frequency) caused a slight increase in the rate of respiration and a sharp rise in blood pressure, which was followed by a fall after the end of stimulation. Second stimulus, at a much lower frequency, caused only a slight, delayed rise in blood pressure; and the third stimulus, at a still lower frequency, caused no apparent disturbance during excitation, but was followed by a rebound fall in blood pressure. The electrodes were in the anterior hypothalamic area just caudal to the optic chiasm.

with pupillary constriction, while high frequencies (50–1600 per sec.) usually caused the pupils to dilate. The nature of the response appeared to depend upon the frequency of stimulation, rather than upon the position of the electrode in the hypothalamus. If the electrode were in the optic tract or the oculomotor nerve, changes in the stimulating frequency caused only a change in the size of the pupillary response, without once altering its sign.

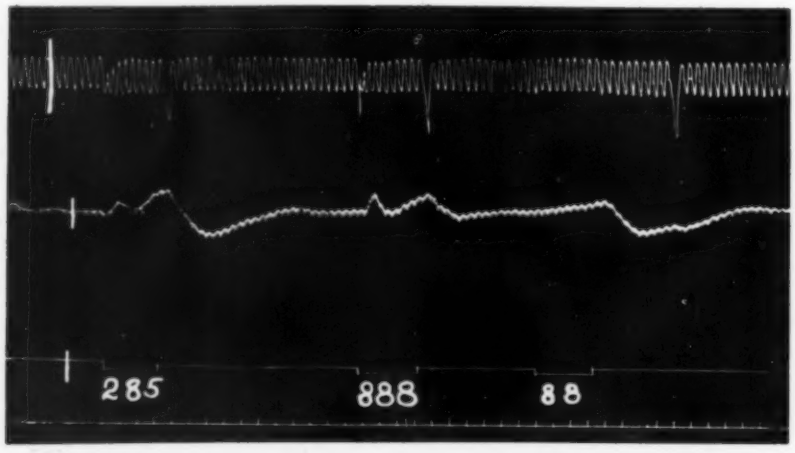

FIG. 4. A record similar to Fig. 3, except that the highest frequency shows only the pressor response. Since this is a part of a long continuous record, reference points, equidistant from the starting points, have been added in white ink. The electrodes were in the posterior hypothalamic nucleus.

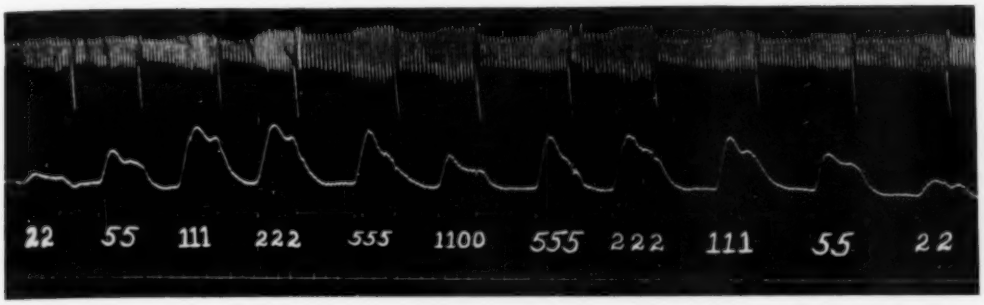

FIG. 5. Consecutive records taken without stopping the kymograph, showing variation in magnitude of pressor and respiratory responses with different frequencies. Optimum frequency range for pressor response is between 55 and 555 impulses per sec. Repetition of same frequency produces similar responses. The electrodes were in the rostral end of the ventromedial hypothalamic nucleus.

DISCUSSION

Pressor and depressor responses were obtained from both the anterior and posterior hypothalamus; the nature of the response was often dependent upon the frequency of the stimulus. Bronk, Pitts and Larrabee (5) have observed a fall in blood pressure during hypothalamic stimulation of a frequency of 2 per sec. associated with a cessation of electrical activity in an efferent sympathetic nerve, the inferior cardiac. Stimulation of the same hypothalamic area at the rate of 20 per sec. caused an increase in the blood pressure and an increased sympathetic activity. If central or peripheral stimulation may inhibit the vasopressor apparatus, it seems improper to conclude from a fall in blood pressure that a "parasympathetic" nerve or center has been excited. Since dilatation of the pupil may occur reflexly in the absence of any innervation from the thoraco-lumbar division of the autonomic system (6, 7, 8), a widening of the pupil during hypothalamic stimulation may be an expression of decreased activity of the oculomotor

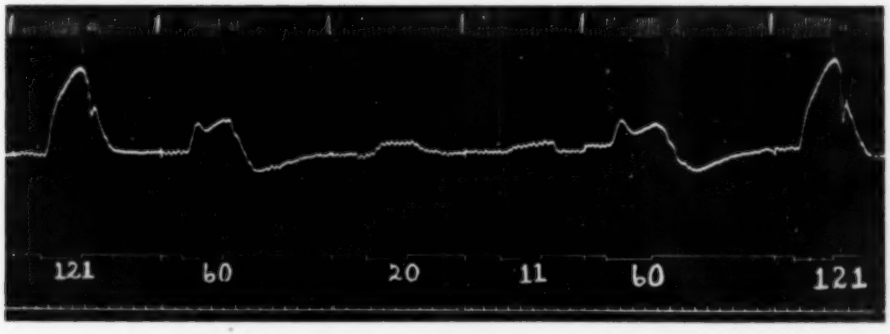

FIG. 6. Compare first stimulus with last, and second with fifth. Discontinuous record. The electrodes were in the anterior portion of the hypothalamus near the rostral end of the dorsal hypothalamic area.

nerve. In these experiments one and the same area produced both "sympathetic" and "parasympathetic" responses when activated with stimuli of constant voltage and wave form, but of different frequencies.

The importance of the frequency of stimulation in determining the response is not peculiar to the responses of the visceral effectors. Somatic responses are just as profoundly affected; threshold, facilitation, fatigue, inhibition, and type and magnitude of postural responses, whether elicited from the cerebral cortex or the diencephalon, were all influenced by the fre-

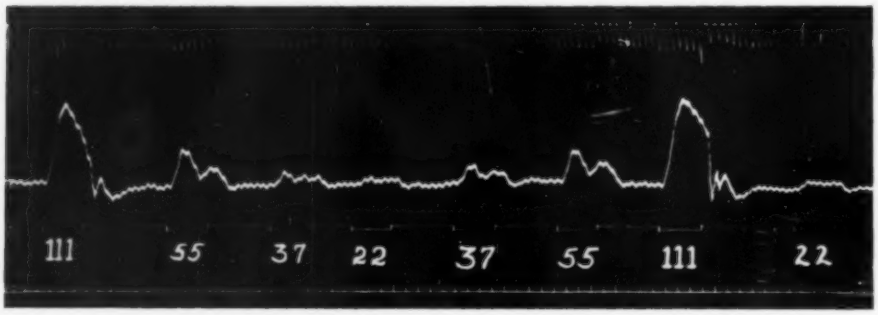

FIG. 7. Similar to Fig. 6, but continuous. The area stimulated was dorsal to the optic chiasm, between the nucleus ovoideus and the supraoptic (tangential) nucleus.

quency of excitation (9). The only somatic movements graphically recorded in the present experiments were respiratory. In almost every case the rate and depth of respiration changed when the frequency of the stimulus changed. In Fig. 2, a low rate of stimulation decreased the respiratory rate; a higher frequency of stimulation caused an acceleration of respiration.

The responses to stimulation of some areas in the hypothalamus could be reversed in sign by altering the frequency of the stimulus (Fig. 2); other areas yielded responses which could be altered only in magnitude (Fig. 5). Reversals of response in this series of experiments occurred often enough to raise a question about strict localization within the hypothalamus of areas which are designated as pressor, depressor, pupillary dilator, or pupillary constrictor.

SUMMARY AND CONCLUSIONS

1. In most cases the magnitude of the pressor and respiratory responses increased with frequencies up to several hundred per second. Beyond this optimum, further increases in frequency caused a progressive diminution of the response. This optimum varied from one part of the hypothalamus to another and from cat to cat.

2. Neither facilitation nor fatigue was responsible for the changes in response. An entire series of responses to stimulation of one area could be repeated immediately or after half an hour and each frequency would elicit

its characteristic response. The sequence in which the stimuli were applied in a series did not affect the response.

3. Differences in spread of current at different frequencies do not account for the change of response.

4. In some instances, changes of frequency altered the character of the response.

5. Alterations in the frequency of stimulation caused changes in the visceral responses in cats in which the skeletal muscle had been paralyzed by curare.

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EXPERIMENTAL STUDY OF GASTRIC ACTIVITY RELEASED FROM CORTICAL CONTROL

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NUMEROUS clinical and experimental studies have dealt with the influence of the cerebral cortex upon the activity of smooth muscle. Fulton (1938) has made an excellent review of the subject. In the recent symposium by Langworthy *et al.* (1940) on the physiology of micturition, the cerebral cortex was considered in relation to vesical activity. However, few viscera besides the bladder are so readily adaptable to investigative procedures.

Although easily accessible, the gastro-intestinal tract, particularly the stomach, is known to be regulated by the interaction of a great complexity of mechanical, chemical and neurogenic factors. Nevertheless, certain clinical observations have suggested an influence by the cerebral cortex over gastro-intestinal movements. Stomach complaints develop frequently in anxiety states and other disorders at higher levels of integration. Watts and Frazier (1935) asserted that the abdominal aura often preceding epileptic convulsions coincides with abnormal gastro-intestinal motility. Robinson (1939) concluded from the radiologic examination of 100 patients that there is no characteristic gastro-intestinal pathology in epilepsy but did not study gastric motility in relation to convulsions. Definite gastric phenomena, particularly the syndrome of morbid hunger and restlessness as described by Levin (1936), are known to occur in relation to brain tumors, cerebral degenerative disease, trauma and other processes damaging the frontal cortex. Levin (1935) discussed the same condition associated with cortical diplegia in children.

Experimental data derived from stimulation and ablation procedures have further indicated a control of gastro-intestinal activity by the cerebral motor and premotor cortices. Bochefontaine (1876) first produced gastric contraction, pyloric relaxation and increased peristalsis in the dog's small intestine and colon by stimulating the sigmoid gyrus. His results have been corroborated by recent workers, particularly Watts (1935) who gave evidence that cortical representation for the gastro-intestinal tract contains both excitatory and inhibitory components. Watts and Fulton (1935) delimited the more excitable cortical foci to area 6 in monkeys. They (1934) also showed that bilateral partial or complete ablation of the frontal lobes caused morbid hunger and occasionally intussusception. Mettler *et al.* (1936) used roentgenographic methods to demonstrate disturbances in gastro-intestinal function in the cat after localized ablations of cerebral cortex.

Although previous clinical and experimental evidence is significant and the nervous regulation of gastro-intestinal activity has been investigated by

various methods, the influence of the cerebral cortex on gastric motility has never been demonstrated graphically. Langworthy and Kolb (1935) produced a condition simulating pseudobulbar palsy in cats by removing both cerebral motor cortices. Their animals subsequently developed general overactivity and ravenous appetites. The present paper entails a graphic study of the alterations in gastric motility in such preparations.

METHOD

A simple yet constant and reliable recording device was utilized (Fig. 1). A light rubber balloon, fastened over the tip of a small soft rubber catheter (14 French), was introduced through the mouth into the stomach. The catheter was connected to a water reservoir in

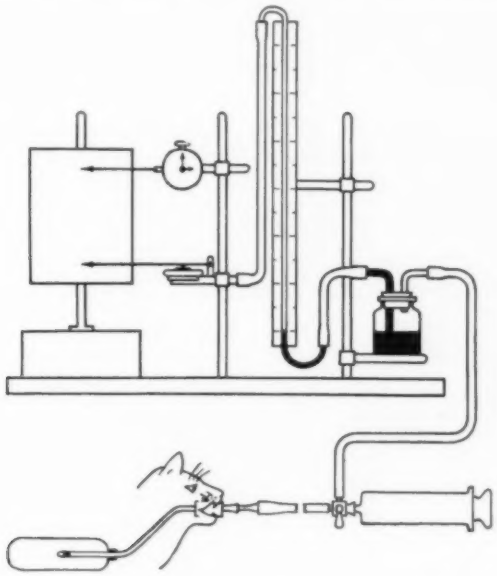

FIG. 1. Diagram of the gastrometric apparatus.

such a way that fluctuations of air pressure in the stomach balloon would register on the metric scale of a water manometer attached to the reservoir. Corresponding variations in the air column over the manometer fluid were recorded by tambour on a kymograph. Time marks and pressure-volume values were included on the tracings. The stomach was filled with air* in 20 cc. increments at appropriate intervals. This was accomplished by means of a syringe and 3-way stop-cock inserted in the system between catheter and water reservoir. Since the balloon filled the entire viscus, the pressure gradients as recorded graphically represented a constantly changing summation of all smooth muscle activity in the stomach wall. Hence it was necessary to recognize total responses rather than individual peristaltic movements.

* In their studies on micturition, Langworthy and his coworkers used water to distend the bladder. This method approached physiological conditions and was useful in observing sphincter activity. However, since the stomach has two openings, it is necessary to confine the distending substance in a balloon if the stomach is to be left *in situ*. Water was tried in this balloon method but its weight on the stomach wall dampened the recording process. Air, acting as a pressure-transmitting medium against a water column, was found to afford maximal sensitivity to minute changes in intragastric pressure.

In order to obtain satisfactory records, it was found convenient to immobilize the animals. Those forcibly restrained while conscious struggled so that the tracings were unsatisfactory. Anesthesia with ether or nembutal either subdued or completely abolished stomach activity. Finally, bulbocapnine† produced a cataleptic-like state in the conscious experimental animal without interfering significantly with peristalsis. This drug, which was used during all gastrometric readings in the present study, had been employed previously by Kolb and Langworthy (1938) in studies on micturition and is admirably suited to such purposes. About 100 mg. of bulbocapnine subcutaneously were sufficient to quiet a 2 to

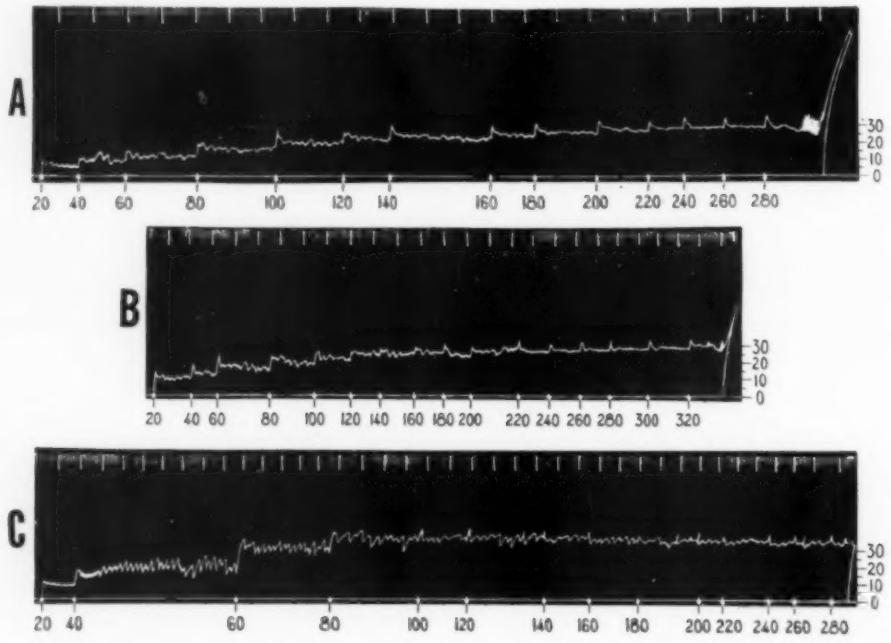

FIG. 2. Kymographic tracings of stomach activity in one of our preparations before and after successive removal of the motor cortices. The upper record (A) was made from the normal animal, the middle (B) followed ablation of the motor cortex on one side and the lower (C) was made after removal of the second cortex. Pressure values in centimeters of water are placed at the end of each record. Volume is indicated in cubic centimeters of air where each increment was introduced. Time is marked in five-second intervals accentuated every minute.

3 kg. cat. Some animals tended to cry while under the influence of the drug and were best studied blindfolded in absolute quiet.

Records were made from the normal animal and at appropriate intervals after unilateral and bilateral removal of the motor cortices. All operations were performed aseptically under nembutal anesthesia. Full recovery from shock and debilitation was permitted between operations and before gastrometric readings.

EXPERIMENTAL FINDINGS

Studies were made upon 6 cats; representative records and composite results are given. Figure 2 consists of a series of typical tracings from one of

† Bulbocapnine was obtained through the kindness of the Merck Co., Inc., Rahway, N. J.

our animals. Volume increments are designated on the baseline in cubic centimeters of air and pressure values are indicated at the end of the records in centimeters of water. Time is marked in 5-second intervals accentuated every minute.

The first graph (A) was made from a normal animal. It will be seen that the intragastric pressure, which rose to 5 cm. of water after the first 20 cc. increment, increased quickly to 15 cm. during the subsequent four increments. The pressure then continued to rise slowly until the limits of stretch were reached at 260 cc. of air and 28 cm. of water. After the introduction of the next 20 cc. of air the pressure fell slightly. A minute later the cat drooled, retched and vomited. Waves of gastric contraction occurred throughout the record but were most intense in the early stages of filling and hardly perceptible toward the limits of stretch. The waves were irregular, averaged 3 or 4 to the minute and never exceeded 5 cm. of water in amplitude. The stomach accommodated promptly to the sudden stretch of each added increment of air.

The second tracing (B) was obtained at a suitable period after the removal of one motor cortex. It shows a slightly earlier and higher rise of intragastric pressure than the first record. After 100 cc. of air had been introduced into the stomach, the pressure remained at 20–25 cm. of water until filling was nearly complete. When the last increment, totalling 320 cc., was injected the pressure rose to 30 cm., but, as before, it fell slightly within the next minute and the animal retched and vomited. An occasional increase in the amplitude of gastric contractions was evident with no essential change in their frequency or duration. In some instances, slowness in accommodation by the stomach wall to added increments suggested a response to stretch. This could be seen especially after the third and fourth additions of air.

The third record (C) followed removal of the second motor cortex. The cat had recovered fully from the operation with characteristic overactivity and ravenous appetite. As filling progressed, tonus in the stomach wall was greater than ever before, and past the 100 cc. level the pressure remained around 35 cm. of water. The experiment was concluded at 280 cc. although no retching occurred. A marked increase in the amplitude of gastric contractions was evident soon after the second increment when a distinct elevation of tone accompanied the onset of activity. The waves fluctuated as much as 12 to 14 cm. of water, and this activity persisted to the end of the record with only slight reduction in amplitude. There was no change in the form or frequency of the waves.

In Fig. 3, the top tracing (A) was made from an intact animal and the middle tracing (B) from the same animal after removal of both motor cortices. The first record is similar to that in Fig. 2 except for a stomach capacity of 500 cc. and more marked peristaltic activity earlier in filling. After operation, the tone baseline during accommodation was distinctly higher with increased amplitude and persistence of peristalsis. Most striking

was the delayed relaxation following each addition of air. In some instances, as after increments 80, 120, 140, 160 and 180, the tone baseline dropped momentarily to regain its former level within the next 30–40 sec.

The lower three tracings in Fig. 3 represent activity in the oesophagus of one of our preparations. After the gastrometric reading, the stomach balloon

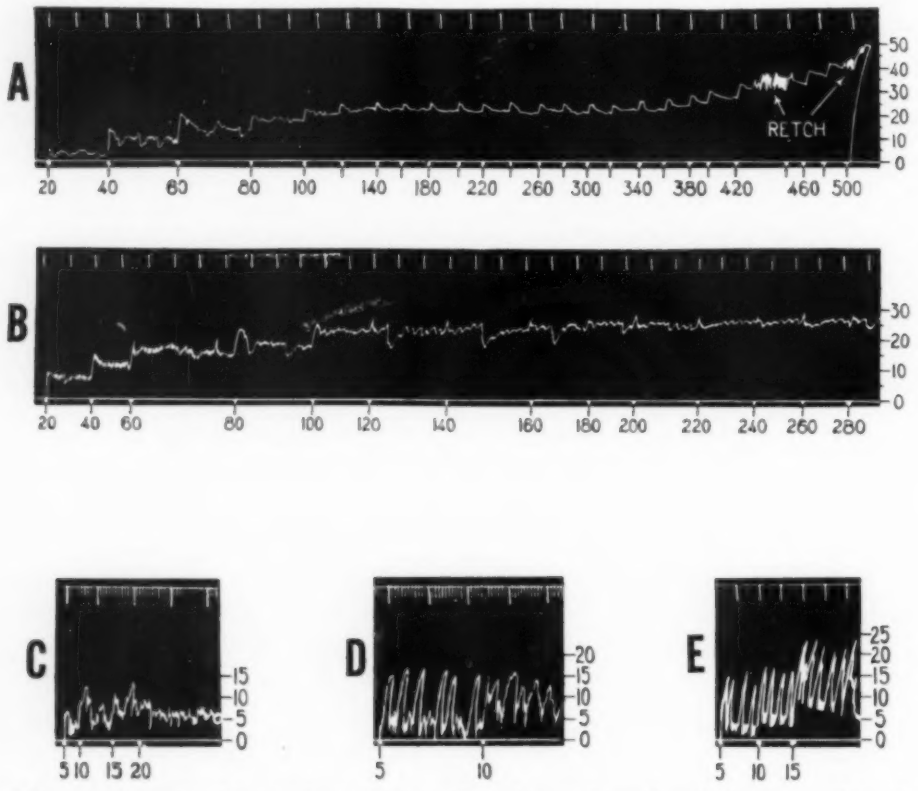

FIG. 3. Kymographic tracings of stomach and oesophageal activity in another of our preparations before and after successive removal of the motor cortices. The upper record (A) was made from the normal animal. The middle tracing (B) was obtained after removal of both motor cortices and shows unusual types of wave activity. The lower three tracings were made from the same animal by pulling the stomach balloon into the oesophagus. The first record (C) was made from the normal animal, the second (D) after ablation of the motor cortex on one side and the third (E) following removal of the second cortex. Pressure, volume and time values are indicated as in Fig. 2.

was deflated, pulled into the oesophagus and reinflated with air in 5 cc. increments. The first record (C), made from the normal animal, showed irregular contraction waves averaging 2 or 3 per min. They never exceeded 8–10 cm. of water in amplitude and disappeared after 20 cc. of air had been injected. The tone baseline averaged 5 cm. of water. In the second record (D), obtained after removal of one motor cortex, contractions were more regular and increased in amplitude to 10–12 cm. with no change in frequency or baseline. The third graph (E), made following ablation of the second cor-

tex, demonstrated even greater oesophageal contractions of 12–15 cm. recurring regularly at the same rate throughout filling. There was also a slight baseline elevation to 8 cm. of water after the third increment. During all oesophageal readings, considerable traction on the catheter was required to prevent contraction waves from forcing the balloon into the stomach.

Arranged similar to the records in Fig. 2, a diagrammatic representation of the changes in gastric activity occurring after successive removal of the motor cortices is given in Fig. 4. The schematic tracings are patterned after an analysis of studies on 6 cats in an effort to illustrate the increase in tone

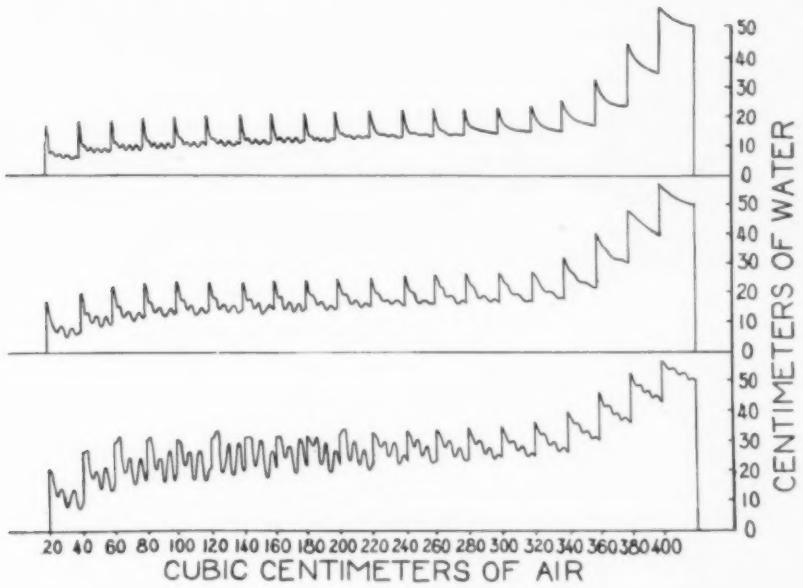

FIG. 4. A diagrammatic representation of the changes produced in gastric activity by successive ablation of the cerebral motor cortices. The schematic tracings are patterned after an analysis of our studies on six cats in an effort to illustrate changes which occur in tone, wave activity and response to sudden stretch. The upper diagram represents these factors in the normal animal, the middle illustrates changes following removal of one motor cortex and the lower shows the result of ablation of the second cortex.

level, the greater amplitude and persistency of gastric contractions and the slowness of accommodation to added increments which took place in our preparations.

The normal stomach responds to stretch by active reflex relaxation to an appropriate tone level. The stomachs of these animals varied considerably in tone, motility and response to stretch. The typical pressure curve rose abruptly during initial filling and slowly or not at all throughout accommodation even though the stomach volume increased steadily. The curve remained fairly constant for each animal and was marked by small irregular waves most pronounced early in filling and inclined to disappear during accommodation or at the limits of distension. At the limit of smooth muscle

elongation, the pressure curve rose quickly again as the connective tissue network limited further stretching of the stomach wall. This point was usually ushered in by drooling and terminated by vomiting. There were occasional minimal responses to sudden stretch characterized by a brief rise in pressure immediately after the introduction of an increment of air. The initial steep rise in pressure usually leveled after the introduction of 60 to 80 cc. of air. From this point on the pressure averaged 10 to 15 cm. and wave fluctuations 1 to 3 cm. of water. Since the pressure rose rapidly at the limits of distension, readings were discontinued after the pressure reached 50 cm. of water. Stomach volumes varied from 300 to 500 cc. at this level.

Changes in gastric activity were evident in some instances, as described above, after the removal of one cortex. In general, however, they were inconstant.

Following removal of the second cortex, most of the recovered animals exhibited restlessness and ate greedily. Stomach records made at this time revealed a distinctly altered wave pattern marked by greater amplitude, regularity and persistence of excursions. Tone in particular was increased throughout filling, especially at the height of wave activity. This usually began early in the period of expansion and continued well into the limits of distension. Distinct and consistent responses to sudden stretch followed each added increment. Pressure rose more quickly during initial expansion and averaged 20 to 25 cm. of water during accommodation. Wave fluctuations varied between 10 and 20 cm. and at times showed a tendency to periodicity. Stretch responses caused additional temporary and sometimes prolonged pressure elevations of 3 to 5 cm. No changes in stomach volume were evident.

After removal of both cerebral motor cortices there was less tendency to retch and vomit at the limits of distension, but drooling would occur as usual. The animal would often remain quiet and sometimes sleep in a plaster cast during the reading without the administration of any drug. Under these circumstances, periods of skeletal muscle activity manifested as running movements of the legs would develop at the onset of powerful waves of gastric contraction. The cat would become quiet again as the waves subsided. This phenomenon suggests a correlation between the increased spontaneous activity produced by frontal lobe lesions, studied quantitatively by Langworthy and Richter (1939), and a state of gastro-intestinal hypermotility.

Discussion

Nervous control of smooth muscle activity in different hollow viscera varies in accordance with the demands placed upon them. It has been shown that the bladder stores quantities of urine at relatively low pressures, whereas eventually a sustained contraction of the vesical muscle empties the organ completely. The stomach in its entirety has two functions, the storage of food by appropriate relaxation and the trituration of food by

rhythmic peristaltic action. The latter also causes progressive emptying when it is properly synchronized with the activity of the pyloric musculature. These functions are coordinated reflexly with the degree of filling, the character of the gastric contents and outside influences mediated through control by the central nervous system.

Our experiments record graphically definite alterations in gastric tone and motility following ablation of the cerebral motor cortices in the cat. The demonstrable production of greater persistency, consistency and strength of stomach contractions and increased tone throughout distension is unmistakable evidence of release from a regulating influence by the motor cortex. The apparent development of a marked stretch reflex with delayed relaxation of the stomach wall after sudden distension is confirmatory.

The results of this study correlate in some degree with earlier studies by Langworthy and Hesser (1936) on micturition released from cerebral control. The effect of removal of the cerebral motor cortices as an increased response of the vesical muscle to stretch was not so well demonstrated in their preparations. This was probably due to a retarding effect by the nembutal used during cystometric readings. However, it was clear that the vesical capacity was reduced after extirpation of one motor cortex and further decreased following removal of the second cortex. The bladder practically lost its ability to accommodate increasing quantities of fluid at low pressures. This development apparently resulted from increased tone in the bladder wall which raised the pressure to the emptying point more quickly past a weakened external sphincter.

Although the method employed in the present study could not demonstrate the emptying point of the stomach, the increase in tone and peristaltic activity following ablation of the motor cortices might be considered, by analogy, conducive to faster emptying and a smaller stomach capacity. Watts (1935) reached similar conclusions in his studies on monkeys. Increased peristalsis would certainly result in a quickening of hunger stimuli which, already lacking certain cerebral retardation, would aggravate the manifestations of great restlessness and ravenous appetite.

Mosso and Pellacani (1882) first demonstrated the peculiar ability of the urinary bladder to hold different quantities of fluid at approximately the same pressure. We wish to emphasize the similar characteristics of the stomach filled progressively with air as shown in the present study.

Of interest was the tendency of the normal as well as the decorticate animals to drool, retch and vomit at the limits of stretch. That the inclination to the latter two was somewhat diminished after removal of the motor cortices would seem to indicate a cerebral influence on the initiation of the reflex act of vomiting as it is integrated at lower levels. The afferent stimuli responsible for the initiation of retching and vomiting seem to have been derived from the state of tension developed in the confining connective tissue network of the stomach wall at the limit of stretch.

It is significant to note that changes similar to those in stomach wall

activity took place in the oesophagus as well. These developed particularly as increased wave amplitude, regularity and persistency and as a slight elevation of the tone baseline. It was apparent, however, that oesophageal activity was of a specialized type designed to propel food into the stomach. Powerful individual contraction waves progressed down the oesophagus at more or less regular intervals to cause strong traction on the expanded balloon. This was frequently forced into the stomach in spite of efforts to hold it back. Since the balloon extended almost the entire length of the oesophagus, it was impossible to distinguish smooth muscle activity in the lower oesophagus from that of striated muscle in the upper oesophagus. Nevertheless, these demonstrations not only serve to indicate a similarity in the innervation of the smooth muscle of oesophagus and stomach but also emphasize a basic responsiveness of smooth muscle to alteration in its nervous regulation regardless of its special function as part of an organ system.

Our experiments suggest, then, that the smooth muscle of the stomach and oesophagus is controlled by reflex pathways in the nervous system similar to those which control tone and contraction in the vesical muscle. It is probable that the tone and contraction are dependent primarily upon afferent stretch stimuli arising in the muscle itself. This has already been shown to hold true for the bladder. The stretch reflex in bladder and stomach alike is not always evident in the normal animal but can be demonstrated easily after cortical control is removed.

Smooth muscle is represented functionally at higher levels of integration and not necessarily in terms of sympathetic or parasympathetic influence. The cerebral cortex certainly mediates the power to initiate micturition or to suppress vesical contractions and so postpone micturition. In studies of the bladder, it was shown graphically that certain disorders of function are dependent upon organic disease of the nervous system. Often it was possible to postulate the anatomical lesion that produced the abnormality. It is probable that similar criteria can be established for the stomach.

Micturition is always initiated by contraction of the vesical muscle, whereupon the internal vesical orifice is opened mechanically. Many abnormalities of micturition follow disturbances in the force and duration of contraction in the musculature of the vesical wall and not derangements in the sphincters themselves. The term "sphincter disturbance" is, therefore, incorrect.

It is probable that similar conclusions can be drawn concerning the cardiac and pyloric sphincters of the stomach. It has become clear that relaxation of the pyloric sphincter is related directly to the strength of peristaltic waves in the antrum. The manifestations of "cardiospasm" or achalasia of the oesophagus are perhaps dependent not on failure of the cardiac sphincter to relax but primarily on a defect in the power of the smooth muscle in the lower oesophagus to propel food into the stomach.

Conclusions

Utilizing a balloon-tambour air-water system, graphic studies of stomach activity in the cat were made before and after successive removal of the cerebral motor cortices. Definite alterations in gastric activity followed ablation of the motor cortices and were demonstrable as greater persistency, consistency and strength of stomach contractions along with increased tone throughout distension. This was interpreted as evidence of release from a regulating influence by the motor cortex. A marked stretch reflex with delayed relaxation of the stomach wall after sudden distension was also apparent. In the oesophagus similar changes occurred after operation as increased wave amplitude, regularity and persistency and as an elevation of tone. An attempt was made to correlate the results of the present study with those made earlier on micturition released from cerebral control. It was suggested that the smooth muscle of the stomach and oesophagus is controlled by reflex pathways in the nervous system similar to those which control tone and contraction in the vesical muscle.

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